

Plant–Pollinator Interactions in a Changing Climate

by

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for the degree of Doctor of Philosophy

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Abstract

Climate change is shifting the seasonal timing of many biological events, and the possibility of non-parallel shifts in different taxa has raised concerns about phenological decoupling of interacting species. My thesis investigates interactions between climate, phenology, and pollination, using the plants and pollinators of Rocky Mountain meadows as a study system. Interannual variation in timing of snowmelt since the 1970s has been associated with changes in the assemblages of concurrently flowering species in these meadows, suggesting that plant species differ in their phenological responses to climate. Differences between plants and pollinators in responsiveness to changing climate could, in principle, cause early-flowering plants to flower too early in warm years, before pollinators are active. In fact, I found only transient evidence for pollinator deficits in one early-flowering species (*Mertensia fusiformis*), even in an early-snowmelt year. However, the assemblage of pollinators visiting *M. fusiformis* does change predictably over the season, with likely consequences for selection on floral morphology in years when pollen is limiting. Hence, early- and late-flowering populations may evolve in response to phenology of the pollinator community. Differences between plant and pollinator phenologies appear to be due to generally lower temperature thresholds for development in plants, combined with microclimate differences between the soil and the above-ground nests of some pollinators. Phenological decoupling between plants and pollinators seems

possible but unlikely to be catastrophic, since many taxa possess adaptations to temporally variable environments. Nevertheless, for many species, adaptation to novel climates will entail evolutionary change, and species interactions can influence evolutionary trajectories. For species affected by increasing late-summer drought, earlier flowering may be advantageous. However, in laboratory experiments, bumble bees avoid rare, unfamiliar flower types, causing simulated plant populations to fail to adapt to changing conditions. Overall, my work emphasizes the importance of the interplay between species interactions and environmental change.

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Chapter 1

Context

Industrial development in the last two centuries has had many environmental impacts, but climate change may be the one that casts the longest shadow. Positive feedbacks from warming that is already underway, such as decreasing surface albedo from diminishing ice cover (Notz, 2009) and carbon release from melting permafrost (Dorrepaa *et al.*, 2009; Schuur *et al.*, 2009), are likely to further accelerate temperature increases. Effects of climate change on biological systems are inevitable, and many have already been detected (Walther *et al.*, 2002; Rosenzweig *et al.*, 2007).

In temperate habitats, the most apparent effect of recent warming has been the earlier arrival of spring. Advances in the egg-laying dates of birds and frogs, and in the flowering dates of plants, were some of the first documented biological responses to climate change (Beebee, 1995; Crick *et al.*, 1997; Bradley *et al.*, 1999). Such changes in phenology, it was noted, might be considered adaptive if they succeeded in keeping a species' life history well synchronized with the changes in its abiotic environment. However, if other species were not altering their life histories in the same way, there was a chance that phenological change would disrupt species interactions (Crick *et al.*, 1997).

As the number of long-term phenology datasets has grown, it has become possible to search for general patterns and to compare rates of change among taxa. In a global meta-analysis of phenological changes, Parmesan (2007) found that the magnitude of advancement varied significantly among taxa and functional groups, with amphibians, for example, showing a rate of change almost seven times greater than that of herbaceous plants. These differences suggest that fears about disruptions of species interactions are well founded—particularly if the interactions involve unrelated organisms.

Species interactions in a changing climate

Researchers have looked for “trophic mismatch”—that is, phenological decoupling between trophic levels—in a range of ecosystems, including temperate forests (Visser *et al.*, 1998; Thomas *et al.*, 2001; Both *et al.*, 2009), Arctic tundra (Post & Forchhammer, 2008), freshwater lakes (Winder & Schindler, 2004; Seebens *et al.*, 2009), and the open ocean (Edwards & Richardson, 2004; Koeller *et al.*, 2009). Climate change has indeed affected synchrony between interacting species in certain cases. In some (*e.g.*, Edwards & Richardson, 2004; Winder & Schindler, 2004), this has been because different taxonomic groups in the same habitat use fundamentally different environmental cues to regulate their phenology, and these cues are not equally affected by climate change. For instance, emergence of marine diatoms from resting spores appears to depend on a critical photoperiod, whereas phenology of their zooplankton consumers is temperature-dependent (Edwards & Richardson, 2004). In other cases (*e.g.*, Both *et al.*, 2006; Saino *et al.*, 2009), synchrony has been disrupted because one of the interacting species is migratory and dependent on cues for migration that do not reflect the progression of phenology at the breeding grounds.

There has so far been less attention to how changing phenology might affect interactions *within* trophic levels (*e.g.*, competition, facilitation), perhaps because species within a trophic level seem more likely to have similar phenological responses to the environment. However, some evidence from plant communities suggests that warming, by spreading out the flowering periods of individual plants, could generate gaps in community-wide flowering phenology (Miller-Rushing *et al.*, 2007; Sherry *et al.*, 2007; Miller-Rushing & Inouye, 2009; but see Post *et al.* 2008). It has been suggested that such gaps could constitute new “phenological niches” that might be exploited by invasive species (Sherry *et al.*, 2007; Wolkovich & Cleland, in press), or simply that they might represent a period of resource shortage for pollinators (Memmott *et al.*, 2007; Miller-Rushing & Inouye, 2009). It is not clear whether altered phenologies would also mean an altered set of competitors for the native species in the community.

In general, we know little about the demographic and evolutionary consequences of independently shifting phenologies for the species involved (Miller-Rushing *et al.*, 2010). The study by Both *et al.* (2006) of European pied flycatchers (*Ficedula hypoleuca*) remains one of the few cases in which population declines have been documented and convincingly linked to the

growing temporal mismatch between the birds and their insect food (see also Husby *et al.*, 2009). In some cases, of course, phenological shifts could actually benefit one or more of the interacting taxa: from the perspective of the birds' prey, a "mismatch" would seem highly desirable. In contrast, positive effects seem less likely to result from disruptions in mutualistic relationships, in which, one would imagine, both parties benefit from some degree of synchrony.

This has led numerous authors to suggest that pollination might be adversely affected by climate change (*e.g.*, Price & Waser, 1998; Hughes, 2000; Dunne *et al.*, 2003; Cleland *et al.*, 2007; Memmott *et al.*, 2007). Two studies provide support for this idea: Kudo *et al.* (2004) documented a decline in seed set of bee-pollinated spring ephemerals, but not of co-occurring fly-pollinated plants, in one unusually warm year in Japan. They suggested that the early snowmelt in that year had allowed flowering and fly emergence to occur especially early, without a concomitant advance in bee phenology, which is presumably less sensitive to timing of snowmelt. Although this study was essentially anecdotal and did not present data on whether the bee-pollinated plants were pollen-limited in any year, it is still one of the only ones to link a climate anomaly, via phenological change in plants and pollinators, to a possible fitness cost to the plants. Since then, Thomson (2010) has shown, using a longer-term dataset from Colorado, U.S.A., that pollen limitation in another spring-flowering plant (*Erythronium grandiflorum*) has increased over time, perhaps because of poor synchrony with its bee pollinators. Lambert *et al.* (2010) have shown that both snowmelt and the flowering of *E. grandiflorum* have advanced in this area. However, data on bee emergence and activity are lacking, and it is not clear that climate change is responsible for the observed decoupling.

In addition to the two studies above, which inferred pollinator activity from seed set of plants, there are three published analyses of long-term trends in honey-bee (*Apis mellifera*) phenology in Europe (Scheifinger *et al.*, 2005; Gordo & Sanz, 2006; Sparks *et al.*, 2010). In all three, first bee sightings are strongly correlated with spring mean temperatures. In a related paper with local data on vegetation phenology, Gordo & Sanz (2005) showed that although flowering dates of insect-pollinated trees also covary with temperature, the greater responsiveness of the bees leads to stronger decoupling in warm years—that is, first bee sightings precede flowering by a greater amount. Similarly, the advance in honey-bee phenology observed by Sparks *et al.* (2010) was so dramatic (34 days over a 25-year period) that, they argued, a "pollination crisis" driven by asynchrony would be unlikely.

Thus, the available information on bees is both limited and somewhat contradictory. (One might also question the extent to which honey bees, with their overwintering colonies and history of domestication, are representative of pollinators in general, or even bees in general. For example, honey bee colonies in late winter have active, mature workers ready to forage as soon as air temperatures permit. In contrast, there is no bumble bee [*Bombus*] activity in spring until overwintering queens break diapause and emerge from their hibernation sites; the colony life cycle is annual and workers do not overwinter. Although these bees are confamilial, they experience spring's onset in very different ways.) Long-term datasets on butterflies—also occasional pollinators—are more numerous. The global compilation of phenological trends cited previously showed that, on average, butterfly responses to climate change have been of greater magnitude than those of herbaceous plants, but similar to those of trees (Parmesan, 2007).

Overall, then, some data suggest that insect pollinators are at least as responsive to warming temperatures as the plants they visit, while others suggest that changes in plant phenology risk outstripping those of their pollinators. One conclusion that can be drawn from this is that long-term data on phenology of the world's most important pollinators—bees—are in short supply. This no doubt explains why the number of articles reviewing or modelling the likely effects of climate change on plant–pollinator synchrony (*e.g.*, Hughes, 2000; Cleland *et al.*, 2007; Memmott *et al.*, 2007; Hegland *et al.*, 2009; Fabina *et al.*, 2010; Traill *et al.*, 2010; Yang & Rudolf, 2010) now far exceeds the number of data papers documenting such an effect. Clearly, we need studies of pollinator phenology that are not based on long-term data but that nevertheless permit inferences about climate change impacts. We also need tests of the consequences to plant and pollinator populations of changing phenologies (*cf.* Hegland *et al.*, 2009).

Climate change in the Rocky Mountains

In its 2007 report, the Intergovernmental Panel on Climate Change cautioned that complex topography makes climate forecasts for mountainous areas particularly challenging. It also noted, however, that the same processes that are contributing to rapid warming in the Arctic (notably decreased surface albedo due to reduced snow and ice cover) might also play a role in accelerating warming of mountainous regions (Christensen *et al.*, 2007). In fact, a more recent regional climate model simulation has shown that the topographic complexity of alpine areas

amplifies snow-albedo feedback (Rauscher *et al.*, 2008), making these regions particularly sensitive to warming.

In the Rocky Mountains of western North America, changes in climate over the last century have indeed been complex. In the western U.S. as a whole, temperatures rose by $> 1^{\circ}\text{C}$ between 1949 and the early 2000s (Cayan *et al.*, 2001; Knowles *et al.*, 2006), compared to a global average of $\sim 0.65^{\circ}\text{C}$ over a similar time period (Solomon *et al.*, 2007). Increases in winter and spring temperatures have been particularly pronounced (Mote *et al.*, 2005; Trenberth *et al.*, 2007), and the upward trends are stronger at higher elevations (Diaz & Eischeid, 2007). However, the initial effects of climate change on mountain snowpack were highly dependent on local starting conditions. Rising temperatures tended to decrease snow accumulation in areas where mean winter temperatures were already close to freezing, such that a small amount of warming could change snowfall to rain (Knowles *et al.*, 2006). In contrast, in more inland and higher-elevation areas, warming was insufficient to have this effect. Between 1950 and 2000, in several sites in the southern Rocky Mountains, snowpack actually deepened, owing to a general increase in precipitation that outweighed the effects of rising temperatures (Mote *et al.*, 2005).

At the Rocky Mountain Biological Laboratory (RMBL) in central-western Colorado, where I have conducted my field work, the opposing forces of increasing winter precipitation and rising temperature seemed to have resulted in stalemate by 2000: there had been no change in date of snowmelt at the local weather station since 1975 (Inouye *et al.*, 2000). The warming temperatures appeared to be responsible for certain changes in the animal community (notably, earlier arrival of migratory robins and earlier emergence of hibernating marmots); but the stasis in snowmelt date, with which flowering dates of many species are correlated, meant that there had been no corresponding change in plant phenology (Inouye *et al.*, 2000). This suggested growing asynchrony between consumers and the plants on which they depend. If such trends were to continue, the consequences for communities could be dramatic.

Since 2000, the story has changed. With the majority of the warmest years on record having occurred in the past decade (WMO, 2009), the effects of rising temperatures have come to dominate trends in snow accumulation and snowmelt in the Rockies. The most recent data show that, since the 1970s, snowpack has declined and timing of snowmelt has advanced throughout Colorado, even at high elevations, and particularly in the western mountains (Clow, 2010). There

is growing concern about late-summer drought in the American Southwest, a region where most surface water comes from snowmelt and where summer moisture deficits have already been linked to increasing tree mortality (Stewart *et al.*, 2005; Bigler *et al.*, 2007; van Mantgem *et al.*, 2009; Overpeck & Udall, 2010). In some recent years, deposition of a dark, heat-absorbing dust layer on the surface of the mountain snow has exacerbated the effects of rising temperatures and accelerated melting even further (Painter *et al.*, 2007; Steltzer *et al.*, 2009). Because the dust originates in the increasingly arid southwestern deserts, it is thought that dust storms will increase in frequency in the future (Painter *et al.*, 2007), potentially causing trends in snowmelt date to outpace those in air temperatures. If the timing of flowering were to continue to track snowmelt, these diverging trends could generate the opposite scenario from the one suggested by Inouye *et al.* (2000): plants might begin flowering before animals are active. Under both scenarios, however, climate change is expected to negatively affect synchrony between plants and animals because plants are thought to be more affected than animals by the timing of snowmelt.

Pollinators of Rocky Mountain wildflowers

The animals that matter most for pollination around the RMBL include two common species of hummingbirds (*Selasphorus* spp.), on the order of 100 species of bees (but no honey bees), and many species of anthophilous flies, wasps, and Lepidoptera. The hummingbirds are migratory, but most of the insects are year-round residents. Due to the short growing season at this elevation (~3000 m above sea level), most insects can complete only a single generation per year; however, longer (semivoltine) and shorter (bivoltine) life cycles have been recorded in some aquatic insects (Taylor *et al.*, 1999; Peckarsky *et al.*, 2000).

Bumble bees (*Bombus* spp.) are the most conspicuous flower-visiting insects in the area, and they and the plants they visit have long been popular research subjects at the RMBL (*e.g.*, Inouye, 1978; Price & Waser, 1979; Pleasants, 1980; Zimmerman, 1980; Pyke, 1982; Gori, 1989; Newman & Thomson, 2005; Elliott & Irwin, 2009). Bumble bees have the ability to warm their flight muscles well above ambient temperatures and therefore can be active when weather is too cold for most other Hymenoptera (Heinrich, 1979a; Vogt *et al.*, 1994). In many temperate habitats, including those around the RMBL, bumble bee queens are among the first pollinators to be active in spring. They and their workers, which are active from midsummer on, visit a wide

range of plant species and are undoubtedly important pollinators. Although some studies have shown correlations between daily maximum temperatures and bumble bee emergence (Alford, 1969; Szabo & Pengelly, 1973), their underground nests presumably constrain bumble bees to emerging sometime after snowmelt (*cf.* Inouye, 2008).

Other bees have a very different life cycle from that of bumble bees. Whereas social bees require floral resources throughout the growing season to support colony growth, most bees are solitary and have a shorter flight season—and a correspondingly greater potential to specialize on a small number of plants as pollen sources. Most bees require warmer ambient temperatures for flight than do bumble bees, although some larger species of *Osmia* (Megachilidae) are capable of impressive thermogenesis and can be active at air temperatures as low as 10°C (Stone & Willmer, 1989; Vicens & Bosch, 2000). Also, although many solitary bees (*e.g.*, Halictidae, Andrenidae) nest and overwinter underground, like bumble bees, others (*e.g.*, many Megachilidae) use abandoned beetle burrows in dead or dying trees, frequently well above ground. This would expose these insects to a different microenvironment than that experienced by plants in the same habitats; hence, the risk of asynchrony with plant phenology seems greater for this subset of pollinators. Unfortunately, although cavity-nesting Megachilidae are important pollinators of many plants (*e.g.*, Torchio, 1990; Monzón *et al.*, 2004; Bosch *et al.*, 2006), they have been relatively overlooked in pollination studies around the RMBL, and there is almost no published information on their emergence phenology in the wild (a study by Kraemer & Favi [2005] on *Osmia lignaria* in Virginia is an exception).

Thesis outline

This thesis examines ways in which climate, through its effects on phenology, can affect ecological relationships between plants and pollinators. I have focussed on the subalpine meadow communities around the RMBL—both as field sites and as sources of data and ideas—for a few reasons. First, they provide a convenient system for studying variation in phenology across a steep environmental (elevational) gradient. Second, the existence of long-term study plots in these meadows (established by David Inouye, University of Maryland) has given me access to historical data on flowering phenology (the basis for Chapter 2). Third, the concerns about phenological decoupling between plants and animals raised by Inouye *et al.* (2000) and others working in these habitats (*e.g.*, Price & Waser, 1998; Dunne *et al.*, 2003) indicated a need

for deeper study of climatic influences on pollination. Of course, I do not claim that plant–pollinator relationships in these meadows are necessarily generalizable to other areas. In particular, most subalpine plants and pollinators appear to be relatively unspecialized as far as pollination is concerned (*cf.* Moldenke, 1979), while environmental disruptions such as climate change might have the greatest impacts on specialists. However, as noted above, dramatic changes in climate and phenology are being documented in the southwestern U.S.—in the high mountains in particular—and many of these changes have been observed since I began my dissertation research in 2006. This rapid change lends urgency to studies of the impacts of climate warming on high-elevation communities.

Perhaps unusually for a thesis on climate change, I have not used any type of artificial warming structure in my research. In principle, experiments have an advantage over observational studies by permitting more unambiguous attribution of effects to specific causes. However, this is not necessarily the case for warming experiments: Warming structures often simultaneously modify more than one component of the environment (*e.g.*, radiant heat both reduces snowpack and warms soil [Dunne *et al.* 2003]; open-top chambers raise temperatures and reduce wind [Marion *et al.* 1997]); furthermore, it is not clear that these compound effects are a realistic representation of future global warming. Worse, for my purposes, is the small spatial scale of any warming experiment. Despite some limitations, warming structures have been useful for studying plant and soil responses to aspects of climate change. But they do not affect the phenology of animals that nest outside the warmed area and enter only to forage; hence, their utility for simulating species interactions under climate change is limited. I have relied instead on natural climatic variation—both spatial and temporal—along with manipulation of bee nesting habitat, an indoor flight cage experiment, and some computer simulations, to answer questions about how plants and pollinators may be affected by climate change. Climate change is already well under way—that experiment has been done!—and there is much that can be learned simply from interpreting existing patterns.

I begin by examining how interannual climate variation affects phenology of the wildflower community as a whole (Chapter 2). Although several studies have shown that timing of flowering is well correlated with timing of snowmelt in this habitat, none has looked at how changes in the date of snowmelt affect co-flowering patterns—that is, the sets of animal-

pollinated plants that flower concurrently. The identities and abundances of co-flowering species determine the potential for competition or facilitation among plants mediated by shared pollinators, so understanding how these seasonal assemblages shift according to climate is a first step in anticipating climate-change effects on plant–pollinator interactions.

In Chapters 3 and 4, I focus on one plant species in particular (*Mertensia fusiformis*) and examine how its relationship with pollinators depends on timing. This species flowers shortly after snowmelt, at a time when pollinators seem scarce and when the risk of asynchrony between flowering and pollinator emergence seems greatest (*cf.* Thomson 2010). In Chapter 3, I test whether seed set of early-flowering individual plants is pollen limited, and whether this varies over time within seasons or between two years that differed dramatically in the timing of snowmelt. In Chapter 4, I look at how differences in flowering time between nearby sub-populations, driven by small-scale differences in snowpack and snowmelt date, influence the composition of the pollinator community for this plant, and I test whether adaptation to the seasonal change in the pollinator fauna is responsible for among-population variation in floral morphology.

Chapter 5 looks at the extent to which the phenology of flowering can be expected to diverge from that of pollinators. Here, using observational and experimental data on the emergence schedules of cavity-nesting Hymenoptera, I fit functions that predict phenology of several pollinator species from local weather variables. I then compare these to similar functions for flowering phenology developed from data collected at the same sites. My goal in this chapter is to work towards a more mechanistic understanding of the phenology of subalpine plants and pollinators, in the hope of achieving a greater ability to predict which species interactions are most at risk.

Finally, Chapter 6 goes beyond the ecological consequences of climate change and investigates whether certain aspects of pollinator behaviour (specifically, neophobia and frequency dependence in bumble bee foraging) might influence the evolution of flowering phenology. In Chapter 7, I synthesize these findings, make predictions about the likely consequences of climate change for plant–pollinator synchrony, and consider what future experiments and observations are needed to test these predictions.

Chapter 2

Flowering phenology in subalpine meadows: does climate variation influence community co-flowering patterns?

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Abstract

Climate change is expected to alter patterns of species co-occurrence, in both space and time. Species-specific shifts in reproductive phenology may alter the assemblages of plant species in flower at any given time during the growing season. Temporal overlap in the flowering periods (co-flowering) of animal-pollinated species may influence reproductive success if competitive or facilitative interactions between plant species affect pollinator services. We used a 33-year dataset on flowering phenology in subalpine meadows to determine whether interannual variation in snowmelt date, which marks the start of the growing season, affected co-flowering patterns. For two of four species considered, we found a significant relationship between snowmelt timing and composition of the assemblage of co-flowering plants. In years of early snowmelt, *Lathyrus lanszwertii* var. *leucanthus* (Fabaceae), the species we investigated in most detail, tended to overlap with earlier-flowering species, and with fewer species overall. In particular, overlap with the flowering period of *Lupinus polyphyllus* var. *prunophilus*, with which *L. leucanthus* shares pollinators, was significantly reduced in early-snowmelt years. The observed association between timing of snowmelt and patterns of flowering overlap could not have been predicted simply by examining temporal trends in the dates of peak flowering of the dominant species in the community, as peak flowering dates have largely shifted in parallel with respect to snowmelt date. However, subtle interspecific differences in responsiveness of flowering time, duration, and intensity to interannual climate variation have likely contributed to the observed relationship. Although much of the year-to-year variation in flowering overlap remains unexplained by snowmelt date, our finding of a measurable signal of climate variation

suggests that future climate change may lead to altered competitive environments for these wildflower species.

Introduction

Species are shifting their geographic distributions and seasonal timing of reproduction in response to climate warming (*e.g.*, Parmesan & Yohe, 2003; Root *et al.*, 2003; Rosenzweig *et al.*, 2007). Individualistic responses to climate change are expected to produce novel assemblages of interacting species, or “ecological surprises” (Williams & Jackson, 2007). There are now several examples of apparently growing phenological disjunctions between interacting organisms; for instance, between marmots and their plant foods in the Rocky Mountains (Inouye *et al.*, 2000), between flycatchers and their caterpillar prey in Europe (Both *et al.*, 2006), and among three trophic levels within the North Sea plankton community (Edwards & Richardson, 2004). In all these examples, the increase in asynchrony in response to climate change supposedly arises because the seasonal activity patterns of different taxa respond to different kinds of cues. For example, timing of flycatcher migration is apparently dictated by an internal circannual clock, whereas the time of peak caterpillar biomass is determined by springtime temperatures (Both *et al.*, 2006; Visser *et al.*, 2006).

At present there are few examples of shifts in temporal overlap among more ecologically similar species. In the Rocky Mountains of the western U.S., flowering phenology of subalpine plants is thought to be largely controlled by the timing of snowmelt (Inouye *et al.*, 2002; Dunne *et al.*, 2003; Inouye *et al.*, 2003). As a consequence, many species are expected to shift their dates of peak flowering more or less in parallel in response to climatic variation. However, the relationship between snowmelt and flowering onset is stronger for relatively early-flowering species than for later-flowering species (Dunne *et al.* 2003). Furthermore, variation in snowpack and snowmelt date has been shown to influence not only the timing but also the abundance and duration of flowering for certain species. For example, in the midsummer-blooming species *Delphinium barbeyi*, early snowmelt is associated with declines in inflorescence number, which in turn lead to reductions in the plot-level flowering period (Inouye *et al.* 2002). Early snowmelt also reduces the number of flowering *Delphinium nuttallianum* plants per plot and the number of flowers per plant, significantly reducing flower abundance at the population level (Saavedra *et al.*, 2003); however, in this early-flowering species, warming lengthens the flowering period of

individual plants (Dunne *et al.* 2003). It is unclear how temporal patterns of flowering overlap at the community level (co-flowering patterns) might be affected by such variation.

Several authors (Price & Waser, 1998; Dunne *et al.*, 2003; Saavedra *et al.*, 2003) have argued that climate-driven shifts in flowering patterns are likely to affect plant fecundity, because of the potential for co-occurring species to attract and support populations of interacting animals—particularly pollinators (*e.g.*, Lázaro *et al.*, 2009), but also generalist seed predators or florivores. Such complex, indirect interactions are difficult to document in the field, but there is substantial evidence that they occur. For example, the co-flowering of other plants can reduce pollinator visitation to a focal species, as well as the quality of pollen delivered to its stigmas (Waser, 1978; Kunin, 1993; Brown *et al.*, 2002). Competition for pollinators between simultaneously flowering species has been invoked to explain evolutionary divergence in flowering times in biotically pollinated plant lineages (Bolmgren *et al.* 2003). On the other hand, co-flowering plants can be mutually beneficial if the multi-species flowering display attracts more pollinators (Thomson, 1981, 1982; Lavery & Plowright, 1988; Moeller & Geber, 2005)—particularly if the spatial arrangement of the different species minimizes heterospecific pollen transfer (Thomson, 1983). In any case, for an animal-pollinated species, the identity and abundance of other open flowers during (or before) its flowering period may influence its reproductive success (Waser & Real, 1979; Gross & Werner, 1983). Conversely, from the pollinators' perspective, changes in co-flowering patterns may mean that floral resources are scarce at certain times of the year (Memmott *et al.*, 2007), or super-abundant at others.

Here, we use a unique long-term dataset on flowering phenology in subalpine plant communities to determine whether interannual variation in climate affects co-flowering patterns. Specifically, we test whether the timing of snowmelt in a given year is related to temporal overlap in flowering in that growing season. Snowmelt timing is an informative variable because it integrates information on springtime temperatures and winter snow accumulation, and determines both the start of the growing season and availability of soil moisture through the season—both factors that may influence plant growth and flowering (Inouye & McGuire, 1991; Inouye *et al.*, 2002). Furthermore, despite pronounced interannual variability, there has been a trend towards earlier snowmelt in our study area since 1973, corresponding to a significant increase in spring-time temperatures in the area (Miller-Rushing & Inouye, 2009). This trend is expected to continue as the global climate warms: Over the last several decades, increases in

winter and spring temperatures in the mountains of the western United States have led to a lower fraction of winter precipitation falling as snow, and more rapid melting (Mote *et al.*, 2005; Stewart *et al.*, 2005; Knowles *et al.*, 2006; Feng & Hu, 2007). In most of the region, the effects of increasing temperatures are over-riding any increases in snowfall, and, in consequence, the duration of the period of snow cover is projected to continue to decline (Christensen *et al.*, 2007). Understanding community-level responses to variation in the timing of snowmelt is therefore particularly important.

Methods

Study area

Thirty 2 × 2 m permanent study plots were established by one of us (DWI) beginning in 1973–1974 at the Rocky Mountain Biological Laboratory, in Gothic, Colorado (38°57.5'N, 106°59.3'W, 2900 m a.s.l.), and have been monitored for flowering phenology every summer since (except 1978 and 1990). For analyses reported here, we considered data from sixteen unmanipulated plots located in dry, rocky meadows, adjacent aspen forest, and more mesic meadow habitat. Approximately every second day throughout the growing season, typically late May through early September, the number of open flowers (or, for taxa in which counting individual flowers was impractical, such as Asteraceae and Apiaceae, the number of capitulae or flowering stems) of all non-graminoid plants was recorded for each plot. In total, 89 animal-pollinated species were recorded in these plots over the study period.

Total snow accumulation in each winter, and the date of first bare ground in spring, have been recorded since 1975 at a station located in Gothic within 1 km of the plots¹. Snowmelt date varies among individual plots and differs from that recorded at the measurement station according to small-scale topographic and climatic conditions; on average, ground first becomes bare 9.3 days later at the station than at individual plots (s.d. = 8.9 d; data available from 2007 only, D. Inouye, unpublished). However, interannual variation in timing of snowmelt is substantial (range = 21 April–18 June, s.d. = 14.8 d; Fig. 2.1) and exceeds among-plot spatial

¹ Available online at <http://rmbi.org/home/index.php?module=htmlpages&func=display&pid=82>

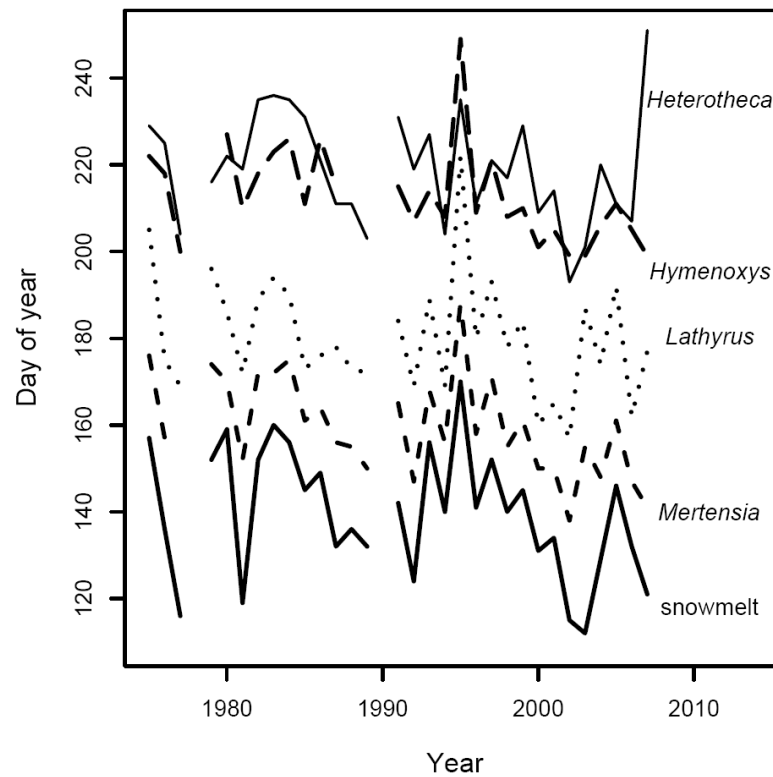


Figure 2.1. Time-series of peak flowering dates of the four focal species and snowmelt date for the period 1975–2007 (data were not collected in 1978 and 1990). Study plots were established beginning in 1973–1974 at the Rocky Mountain Biological Laboratory, in Gothic, Colorado, USA.

variation. In addition, there are air temperature data from the NOAA weather station in Crested Butte, approximately 10 km away and 200 m lower in elevation. We use snowmelt date at the Gothic station as our primary explanatory variable, for reasons mentioned previously. As a check, we also ran analyses using monthly mean temperatures for April, May, and June, but because the inclusion of these variables did not significantly improve our predictive power, we present results based on snowmelt date alone.

Focal species

Plant nomenclature follows Hartman and Nelson (2001); to ease comparisons to older literature from this region, obsolete generic names are given in parentheses. To assess interannual changes in flowering overlap, we considered one particular focal species at a time, and asked how the assemblage of species that co-flowered with that focal species varied across years with the timing of flowering onset. To determine the generality of any effect, we examined four focal species whose flowering periods collectively spanned most of the growing season. These species were selected, a priori, according to the following criteria: (1) they are native, (2) they are primarily bee-pollinated, based on our observations, (3) they are well represented in the dataset, throughout the time-series, in terms of both number of years in which flowering occurred and number of plots in which the species was found, and (4) their peak flowering dates are spread through the season (Table 2.1, Fig. 2.1), such that the flowering period of each focal species reflects a distinct segment of the whole-community flowering season and, therefore, a more or less independent test of the hypothesis. We did not select species that have frost-sensitive buds (*e.g.*, *Delphinium barbeyi*, *Erigeron speciosus*) and therefore show marked reductions in flower number in early-snowmelt years (Inouye, 2008). Even though these species were otherwise suitable for analysis, they might be particularly likely to show a change in overlap patterns in response to snowmelt date owing to this frost-sensitivity. These criteria led us to select the earliest- (*Mertensia fusiformis*) and latest-flowering species (*Heterotheca* [= *Chrysopsis*] *villosa*) for which we had reasonably complete data (*i.e.*, we had missed the flowering peak in 4 of 31 years, at most), as well as two species with intermediate flowering dates (*Lathyrus lanszwertii* var. *leucanthus* [henceforth *L. leucanthus*], and *Hymenoxys* [= *Helenium*, = *Dugaldia*] *hoopesii*).

Table 2.1. Focal species used in analyses and Mantel statistics.

Species	Family	Peak flowering date	No. of plots	Mantel <i>r</i>	<i>P</i>	<i>N</i> (years)
<i>Mertensia fusiformis</i> Greene	Boraginaceae	8 June	14	0.15	0.109	27
<i>Lathyrus lanszwertii</i> Kellogg var. <i>leucanthus</i> (Rydb.) Dorn	Fabaceae	28 June	10	0.22	0.007	30
<i>Hymenoxys</i> (= <i>Helenium</i>) <i>hoopesii</i> (Gray) Bierner	Asteraceae	30 July	4	0.14	0.022	28
<i>Heterotheca</i> (= <i>Chrysopsis</i>) <i>villosa</i> (Pursh) Shinnars	Asteraceae	6 August	7	-0.01	0.497	30

Notes: “Peak flowering date” is the average over all plots and years in which the species flowered. Plots in which the species flowered in at least 15 years were used for analysis of co-flowering communities; the number of such plots is reported. Mantel statistics are shown for correlations between snowmelt date and co-flowering communities (based on complete relative abundance data).

Hymenoxys hoopesii and *Heterotheca villosa* have more similar flowering periods than the other pairs of species but occur in different plots (wetter sites for the former; drier sites for the latter). Of the four focal species, *L. leucanthus* is the one for which we have the most complete data (*i.e.*, it occurs in a relatively large number of plots [10], and the beginning of its flowering period was rarely missed by censuses—something that occurred more often for the more abundant but earlier-flowering *M. fusiformis*). We therefore analyzed patterns of flowering overlap for this species in more detail. Furthermore, *L. leucanthus* is one of three legume species that occur commonly in the study area and that show partial overlap in flowering times. Like *L. leucanthus*, *Lupinus polyphyllus* var. *prunophilus* (“*Lupinus argenteus*” in some previous work from the RMBL) and *Vicia americana* Muhl. ex Willd. are visited by species of *Megachile*, *Osmia*, and *Bombus* (J. Forrest, personal observation; also Pyke, 1982; Gori, 1989); these large-bodied bees are likely the only effective pollinators of these plants (*cf.* Faegri & van der Pijl, 1979). Because of the greater potential for pollinator-mediated interactions among these legumes, we conducted additional, more detailed analyses of patterns of overlap between pairs of these species.

Data analysis

Whole community overlap

We took two approaches to quantifying flowering overlap for our focal species. In the first, we considered the entire assemblage of animal-pollinated plants that were in flower during the flowering period of each focal species to constitute a “co-flowering community” (Fig. 2.2). We then used multivariate statistics to ask whether the composition of this community in a given year (in terms of identity and relative abundances of co-flowering species) varied with the timing of snowmelt in that year.

We quantified the “abundances” of all species in the co-flowering community, including the focal species, by summing all flower (or inflorescence) counts for each species over the relevant time period. Missing individual data points (< 1% of observations) were estimated by linear interpolation. In certain years (1976, 1977, 1992, and 1994), the beginning or end of the flowering season was missed, affecting our calculations of the co-flowering communities for certain species. These years were omitted entirely from analyses involving species’ relative abundances, but were included in analyses that used only presence–absence data. Finally, 1976

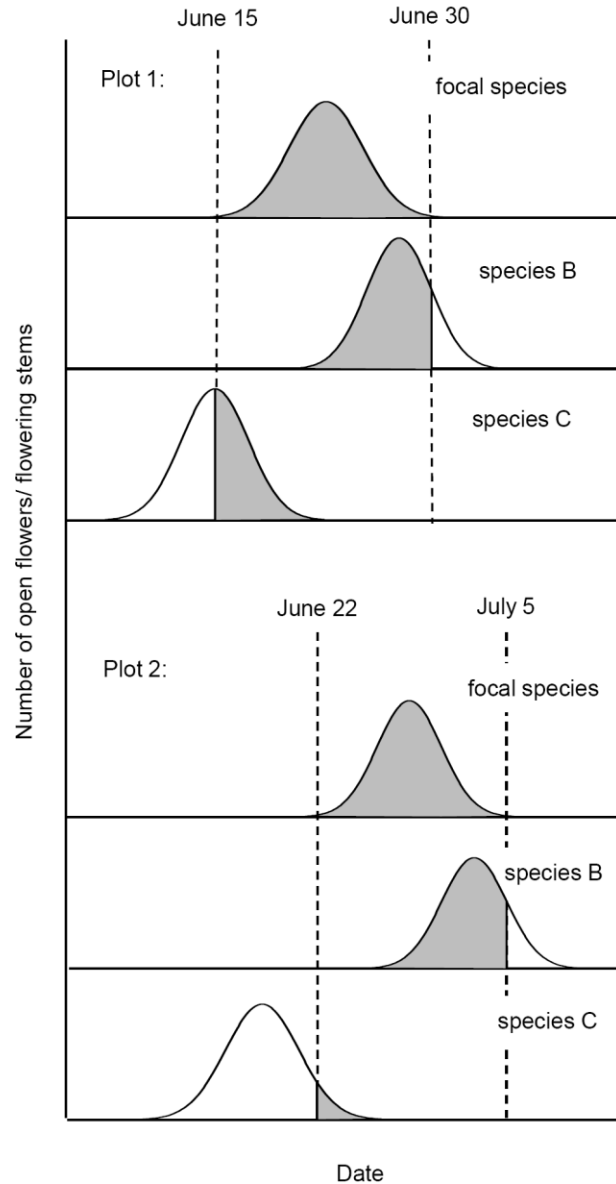


Figure 2.2. Conceptual diagram illustrating how periods of flowering overlap were defined. The figure shows hypothetical flowering curves for three species occurring in two separate plots in one year. The period of flowering overlap for each plot is the time during which the focal species is in flower (the area between the dashed lines); this interval may differ across plots. The “co-flowering community” for the year is the summed floral abundances for all species flowering in all plots within the period of flowering overlap, including the focal species (*i.e.*, the sum of the shaded areas).

was omitted from analyses of the *L. leucanthus* co-flowering community, because the flowering period of that species was missed entirely in the mesic-meadow plots in that year. Some plots have consistently earlier flowering (and snowmelt) than others. Considering the entire flowering period of a species across all plots would therefore inflate estimates of the number of co-flowering species and might obscure patterns related to interannual variation in climate. We instead considered the relevant set of co-flowering species to be those that overlapped with the focal species within each 2×2 m plot, although we recognize that pollinator-mediated interactions between plants are likely integrated over a larger scale than this. Thus, we defined the flowering period of the focal species separately for each plot in each year; however, we summed floral abundances (within the relevant flowering periods) across all plots to construct the co-flowering community for each year (Fig. 2.2). We used only the plots in which the focal species was recorded as flowering in at least 15 of the 31 years.

We used a Mantel test to evaluate the relationship between date of first bare ground and co-flowering community composition across years. That is, we tested for a correlation between two matrices of distances between years: a matrix of differences in snowmelt dates, and a matrix of Bray–Curtis distances between species compositions. Bray–Curtis dissimilarity is given by $\sum_j |y_{1j} - y_{2j}| / \sum_j (y_{1j} + y_{2j})$, where y_{1j} is the abundance of species j in year 1 (Quinn & Keough, 2002). Bray–Curtis distances are more appropriate than Euclidean distances for species abundance data, in which shared zeroes (joint species absences) are common but not necessarily informative (Quinn & Keough 2002). For calculating Bray–Curtis distance, we used relative abundances of all taxa for which we had complete data (*i.e.*, number of flowers of each species or genus as a proportion of all flowers of all species in the plot in that year). We omitted from this analysis species for which we had only presence–absence data, rather than relative abundances, in certain years (*Salix* spp., *Paxistima myrsinites*, *Galium bifolium*). However, we checked the robustness of our results by repeating the analysis, first, with “common” species only (those that flowered in more than one year), and, second, using presence–absence data (common species only) instead of relative abundance. Significance of Mantel correlations was determined by randomization, with 1000 iterations.

To interpret better the Mantel correlation results for *Lathyrus leucanthus*, we used non-metric multidimensional scaling (NMDS), based on Bray–Curtis distances—the same distance measure used in the Mantel tests—to plot co-flowering communities for each year in multidimensional space. The best NMDS solution was found by iteration (400 ×), and significance of each axis was assessed by Monte Carlo randomization. We then plotted the axis scores for each year against the corresponding date of first bare ground to determine whether any of the community axes was related to date of growing-season onset. Note that, unlike other ordination techniques such as canonical correspondence analysis (CCA), the approach we use does not force a relationship between the abiotic variable (snowmelt date) and community composition; rather, it simply allows us to summarize and visualize differences among communities and then independently assess whether these differences are related to variation in climate (Quinn and Keough 2002).

To help explain results obtained in the multivariate analyses, we investigated associations between snowmelt date and flowering variables for the 15 most abundant species in the *L. leucanthus* overlap community. For each species, we evaluated the effect of snowmelt date on peak flowering date, flowering intensity (the maximum number of flowers or inflorescences observed on one date), and flowering duration at the plot level. Flowering intensities were 4th-root transformed to achieve normality. We also tested for an effect of snowmelt date on the total number of species co-flowering with *L. leucanthus*. We verified that there was no detectable temporal autocorrelation in our variables by examining their autocorrelation functions; the absence of significant autocorrelation suggests that treating years as independent data points was justifiable. We conducted both the Mantel tests and NMDS in PC-ORD (McCune & Mefford, 1999). All other analyses were done in R (R Development Core Team, 2007).

Overlap between species pairs

In the whole-community approach described above, each day within the flowering period of the focal species is given equal weight in terms of defining co-flowering community composition, regardless of the abundance of the focal species on that day. For the second approach, we focussed on individual overlapping species and performed a more detailed analysis that took into account the proportion of the total flowering that occurred on each day within the flowering period. As our measure of the flowering overlap between two species, we used Schoener's index

of niche overlap (Schoener, 1970), $SI = 1 - 0.5 \sum_k |p_{ik} - p_{jk}|$, where p_{ik} is the proportion of flowering by species i occurring on day k . SI has values close to 0 when only the tails of the two species' flowering curves overlap and reaches a maximum of 1 when the flowering curves of the two species coincide perfectly. For each year, we calculated SI separately for each plot in which the two species occur; we then averaged the SI values across all plots for each year. Before calculating SI, we filled in values for flower counts on days without data by linear interpolation. We tested for a relationship between SI and snowmelt date using rank-based correlations instead of linear regression because of the large number of zero values. We conducted this analysis for two species pairs that could be expected to interact for pollinators: *Lathyrus leucanthus* – *Lupinus prunophilus* and *L. leucanthus* – *Vicia americana*. We applied a Bonferroni-corrected significance criterion of 0.025 because of the need to conduct the two pair-wise tests separately.

Results

Temporal autocorrelation

We detected no significant temporal autocorrelation in snowmelt date, peak flowering dates of focal species, or the intensity or duration of flowering of *L. leucanthus* ($P > 0.05$), suggesting that individual years may reasonably be treated as independent data points. However, it should be noted that the length of the time-series and the two missing years give limited power to detect autocorrelation.

Whole community overlap

There is a significant Mantel correlation between date of first bare ground and composition of the co-flowering community for two of the four species considered, *L. leucanthus* and *H. hoopesii* (Table 2.1). Obtaining the observed P -values (three of which fall below 0.11) in a set of four independent tests would be highly unlikely in the absence of any real effect (Fisher's method for combining P -values, $\chi^2 = 23.4$, $df = 8$, overall $P = 0.0029$; Quinn & Keough 2002). These results are largely unchanged if we remove from the analysis species that flowered in only one year, but doing so strengthens the pattern observed in *M. fusiformis* (new Mantel $r = 0.18$, $N = 27$ years, $P = 0.055$; the original P -value was 0.109). We also repeated the analyses using presence–absence data—that is, coding any species that overlapped with the focal species as “present” and any that

did not as “absent”—instead of relative abundances. Doing this rendered the pattern for *M. fusiformis* significant (Mantel $r = 0.15$, $N = 31$, $P = 0.031$), because of the increased power afforded by including four additional years in which relative abundance data were unreliable, but did not qualitatively change results for the other species. Non-metric multidimensional scaling of *L. leucanthus* co-flowering communities for all years produced three significant axes, explaining 34.8%, 34.3% and 15.2% of the variation, respectively. Scores on the first axis were significantly associated with date of first bare ground (Fig. 2.3), suggesting that one major axis of variation in community composition is related to snowmelt date. Species with low scores on axis 1 had later peak flowering dates (means across all plots and years; Pearson’s $r = -0.66$, $df = 13$, $P = 0.0080$), indicating that, in years with low scores on this axis (late years), *L. leucanthus* overlapped to a greater extent with later-season species. We detected no significant temporal autocorrelation in axis 1 scores ($P > 0.05$). However, the axis 3 autocorrelation function shows a consistent decrease in the partial autocorrelation coefficient as the lag interval increases, indicating greater dissimilarity among communities separated by larger time intervals; this axis may therefore reflect changes in species abundance over time.

This result suggests that *L. leucanthus* may shift its flowering period disproportionately earlier, compared to other members of the community, in early snowmelt years. *Lathyrus leucanthus* does have a relatively strong response to variation in snowmelt, particularly compared to later-flowering species (see Appendix A). However, individual species have largely homogeneous responses to variation in snowmelt (Fig. 2.4): Although the slopes of the relationship between peak flowering date and snowmelt date differ slightly among species (ANCOVA, snowmelt date $\times$ species $P = 0.089$), this effect is due to a single annual species, *Collomia linearis*, that is relatively unresponsive to variation in snowmelt (Appendix A; Fig. 2.4). The effect of snowmelt date on the *L. leucanthus* overlap community is not influenced by inclusion of this species (Mantel test with *C. linearis* omitted, $r = 0.23$, $P = 0.004$). Thus, a simple examination of shifts in peak flowering across years would not, by itself, point to a relationship between co-flowering patterns and snowmelt date.

The peak number of *L. leucanthus* flowers (summed across plots, 4th-root transformed) was greater in years with later snowmelt (linear regression, $R^2 = 0.16$, $N = 31$, $P = 0.016$). However, mean flowering duration of *L. leucanthus*, although strongly correlated with flowering intensity

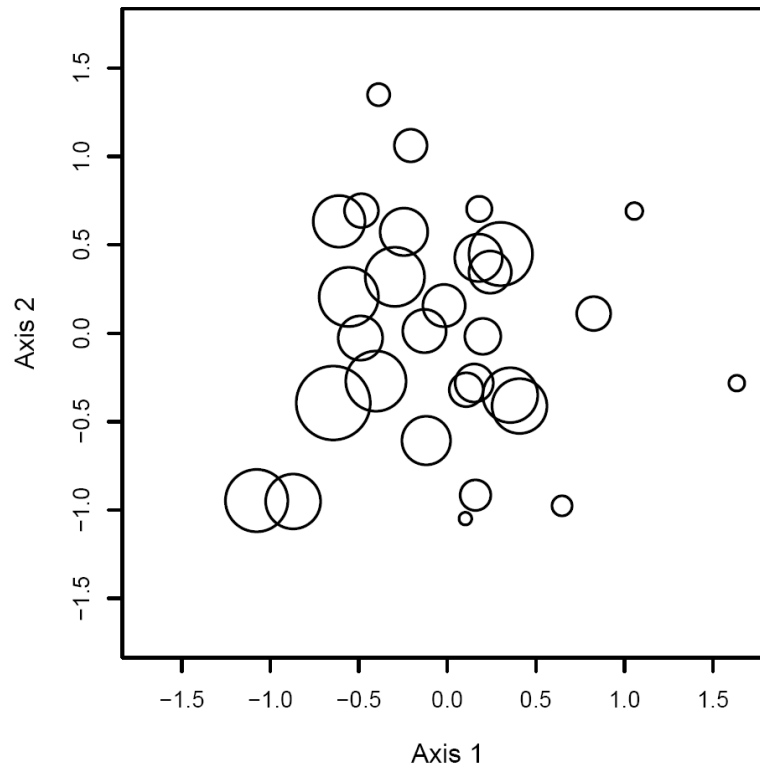


Figure 2.3. Non-metric multidimensional scaling of *L. leucanthus* co-flowering communities (two of three significant axes shown, representing 69% of the variation in community composition). Points in the scaling are individual years; point size is proportional to snowmelt date in that year. There is a negative association between axis 1 scores and snowmelt date ($R^2 = 0.32$, $P = 0.0006$).

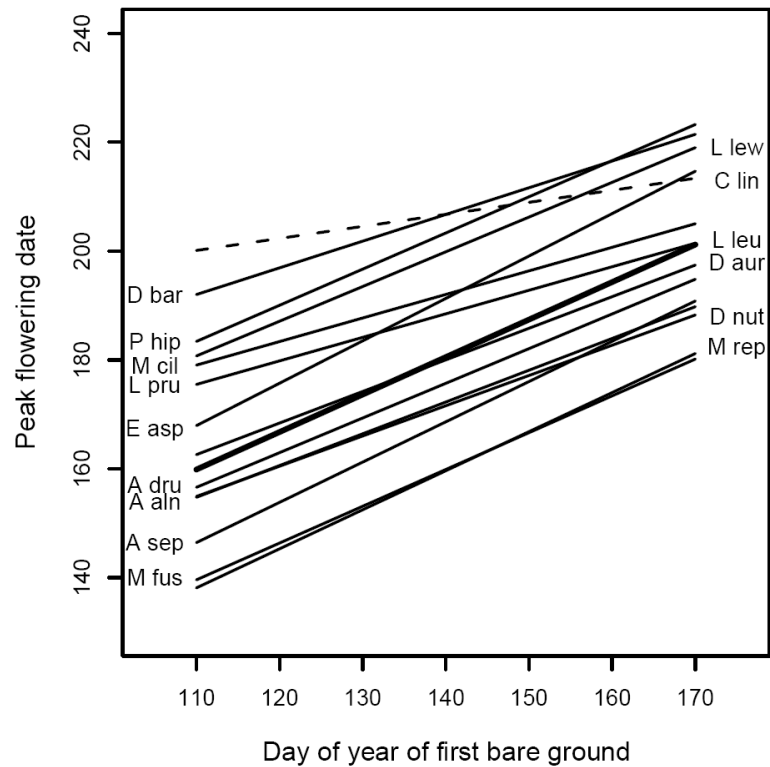


Figure 2.4. Regression lines for peak flowering date vs. snowmelt date for the 15 most abundant species in the *L. leucanthus* co-flowering community. Complete species names are provided in Appendix A. The dashed line represents the annual species *Collomia linearis*; the thicker solid line represents *L. leucanthus*. Individual data points are omitted for clarity.

(Pearson $r = 0.57$, $P = 0.0006$), was not significantly influenced by snowmelt date (linear regression, $R^2 = 0.053$, $N = 31$, $P = 0.11$). In fact, flowering durations of most of the species in this dataset were not strongly affected by variation in snowmelt date (Appendix A). Three of the species that showed the strongest positive association between flowering intensity and snowmelt date are relatively late-flowering species (*Delphinium barbeyi*, *Lupinus prunophilus*, and *Mertensia ciliata*; Appendix A). Greater numbers of flowers of these species in years of late snowmelt might contribute to greater overlap between *L. leucanthus* and later-flowering species in these years. However, because Mantel test results were essentially unchanged when we used presence–absence rather than relative abundance data, it is unlikely that variation in flowering intensity is the sole driver of the patterns we observe. Indeed, variation in snowmelt date also influenced the number of species with which *L. leucanthus* overlapped: There was a positive relationship between snowmelt date and species richness of the co-flowering community (range = 13–34 species; linear regression, $R^2 = 0.12$, $N = 30$, $P = 0.032$), and this relationship remained significant even if only species with more than 10 open flowers or inflorescences per year (over all plots, during the *L. leucanthus* flowering period) were considered.

Despite a trend for earlier melting over the period 1975–2007 (Kendall's $\tau = -0.28$, $P = 0.026$), and a similar trend for earlier peak flowering in *L. leucanthus* over the same time period (Kendall's $\tau = -0.25$, $P = 0.049$), there is no temporal trend in NMDS axis scores ($|\tau| < 0.2$, $P > 0.3$).

Species pairs

Overlap between *Lathyrus leucanthus* and the later-flowering *Lupinus prunophilus* is significantly lower in years of early snowmelt (Spearman correlation between SI and snowmelt date, $\rho = 0.66$, $\alpha = 0.025$, $P < 0.0001$; Fig. 2.5a). In fact, in early years (date of first bare ground < 139), there has typically been no temporal overlap (SI = 0) between the two species at the scale of individual plots, while in later years, overlap values mostly fall between 0.25 and 0.65. Overlap has tended to be less in recent years, but this trend is marginally non-significant (Kendall's $\tau = -0.26$, $P = 0.052$; Fig. 2.5a). There is no relationship between snowmelt date and overlap between *L. leucanthus* and *Vicia*, the last species of the three to flower (Spearman $\rho = -0.07$, $\alpha = 0.025$, $P = 0.72$; Fig. 2.5b).

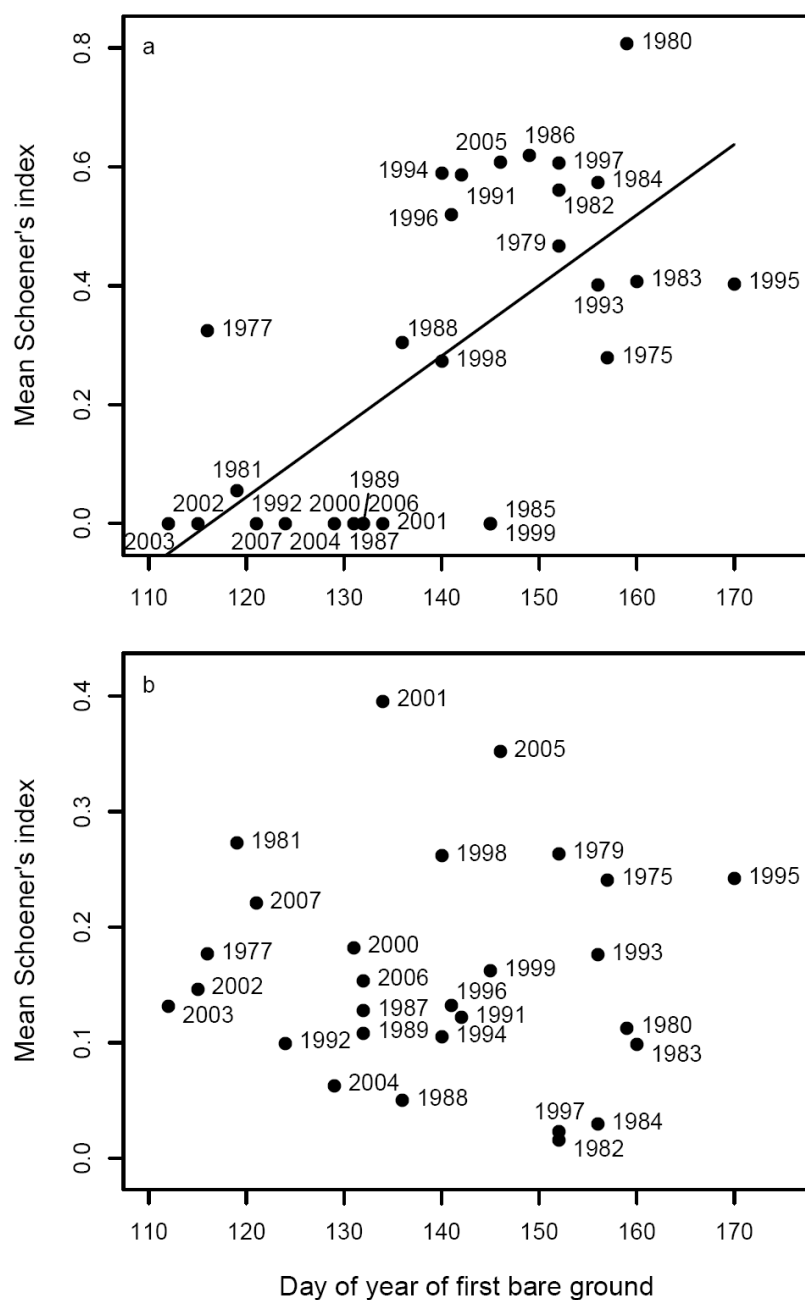


Figure 2.5. Temporal overlap (Schoener's index) between *Lathyrus leucanthus* and (a) *Lupinus prunophilus* and (b) *Vicia americana* as a function of snowmelt date ($N = 30$ years). The regression line in (a) is $y = 0.012x - 1.38$ ($R^2 = 0.42$).

Discussion

For at least two of the four focal species we examined, among-year differences in flowering overlap were partially explained by variation in snowmelt date. In years of early snowmelt, these species flowered together with, and likely shared pollinating animals with, a different suite of other plants than they did in late-snowmelt years. In a third species (*Mertensia fusiformis*), co-flowering patterns may also have been influenced by snowmelt date, but the significance of the effect depended on how we treated the data. Only for *Heterotheca villosa*, one of the latest- and longest-flowering species in these meadow communities, was there no indication of a trend related to snowmelt date. Previous work, both at the RMBL and elsewhere, has shown that flowering times of earlier-flowering species tend to be more responsive to variation in the timing of the start of the growing season (Fitter & Fitter, 2002; Dunne *et al.*, 2003; Miller-Rushing *et al.*, 2007); our results are consistent with the idea that effects of phenological variation can be muted for later-flowering species.

For *Lathyrus leucanthus*, the species we examined in most detail, interannual differences in the assemblage of co-flowering species appeared to be related to a tendency for the species to overlap with more species overall, and to a greater extent with later-flowering species, in years of relatively late snowmelt. In particular, temporal overlap with the later-flowering *Lupinus prunophilus* was greatly reduced in early-snowmelt years. These patterns may be partly due to slight (non-significant) differences among species in the extent to which the date of peak flowering responds to snowmelt timing. At least as important are interspecific differences in the relationship between snowmelt timing and intensity of flowering. As has previously been noted (Inouye *et al.*, 2002; Miller-Rushing & Inouye, 2009), certain mid-summer species, notably *Mertensia ciliata* and *Delphinium barbeyi*, flower more abundantly in years with greater snowpack or later snowmelt. For some species at our study site (*Helianthella quinquenervis*, *Erigeron speciosus*), early snowmelt is accompanied by a high risk of frost damage to buds and a severe reduction in flowering. For these species, there appears to be a threshold snowmelt date, around May 19, before which frost damage to flower buds is very likely (Inouye 2008). Buds of *Lupinus prunophilus* are also frost-sensitive, and flowering can be severely depressed in these early-snowmelt years (Inouye 2008; also personal observations); this helps explain the May 19 threshold we observe in the pairwise overlap with *Lathyrus leucanthus* (Fig. 2.5a)—particularly

if the earliest buds are those most likely to be affected by killing frosts. A threshold effect is not apparent, however, in the community-level analysis (Fig. 2.3).

Idiosyncratic effects of snowmelt timing on flowering duration, with some species flowering for longer periods in late years and some flowering longer in early years, may also explain part of the community-level pattern. Effects on flowering duration were significant for only two of fifteen species considered, however: duration was increased in early years for the relatively early species *Mahonia repens*, but was decreased in early years in the later-flowering, but frost-sensitive, *D. barbeyi*. In contrast to some previous work (spatial gradient study in Price and Waser 1998, Dunne *et al.* 2003), we did not find a general positive effect of early snowmelt on flowering duration for early-flowering species. This is most likely because we considered the flowering period at the plot level, rather than at the level of individual plants—and any individual-level increases in flowering duration could have been compensated for by decreases in abundance of flowering in early-snowmelt years (*e.g.*, in *Delphinium nuttallianum*). In any case, it is clear that the community-level patterns we document here could not be easily predicted from considering species-specific changes in peak flowering dates or flowering intensities alone; instead, they reflect the integrated changes in flowering patterns of multiple species across years and highlight the value of a multivariate approach for understanding community change.

We did not detect strong temporal trends in co-flowering patterns, despite a measurable advance in snowmelt date over this time period. This is partly owing to the great interannual variability in climate, but also to the large amount of variation in flowering patterns that could not be explained by variation in snowmelt timing. Including air temperature data in the matrix of climate variables did not improve correlations between climate and patterns of flowering overlap; but it is possible that other unmeasured or finer-scale climate variables (*e.g.*, snowmelt dates for individual plots) might be important. Gradual demographic changes such as plant growth, death, and recruitment within plots must also contribute to among-year variation in flowering, independent of year-to-year changes in snowmelt date. However, most plants in these communities are long-lived perennials, and there have not been any large-scale changes in species composition during the study period. Losses of flowers to herbivores (*e.g.*, deer) and local frost events may explain some of the remaining variance, but at least the focal species do not appear to be much affected by specialist herbivores. Clearly, predicting future co-flowering patterns would not be straightforward, even if we had reliable local climate forecasts: In addition

to the effects of climate on flowering patterns that we have shown here, there is likely to be a longer-term effect of climate change on the relative abundances of species in these communities (de Valpine & Harte, 2001; Saavedra *et al.*, 2003). Understanding how the phenological and demographic effects of climate change will interact remains a challenge.

We know of no other long-term datasets that would permit an analysis of the type conducted here. A single-season experiment in grassland plots (Sherry *et al.*, 2007) also showed a change in relative flowering times caused by severe (4–5 C°) experimental warming, leading to a midsummer gap in flowering. However, as many species in that system were wind-pollinated grasses, the potential for pollinator-mediated interactions among plants was limited. Spatial variation in patterns of flowering overlap (*e.g.*, across elevational gradients) may be similar to the temporal variation we have dealt with. Among-site comparisons of co-flowering patterns, and their fitness consequences for focal plant species, would be a useful complement to our data.

Presumably, changes in the identities of co-flowering species could influence both the behaviour and population dynamics of flower-visiting insects, leading to altered selective environments for plants (*cf.* Kudo, 2006). However, there are few long-term datasets on North American insects (Williams *et al.*, 2001), and none that we know of with a resolution comparable to the plant dataset we have used here; we therefore lack information on temporal variation (both within and among years) in the populations of what we presume are important selective agents. In the absence of hard data on pollinators, we may imagine some possible consequences of the climate-mediated changes in co-flowering patterns we observe: Reductions in the abundance and overlap of flowers that share pollinators (*e.g.*, *Lathyrus leucanthus* and *Lupinus prunophilus*) could reduce interspecific competition among plants for those pollinators. Alternatively, it could lead to a failure to attract and maintain local populations of generalist pollinators. For example, the presence of flowering individuals of one species can increase pollinator visitation and seed set in a second species (Lavery, 1992; Moeller & Geber, 2005). This effect is possible if the first species is more attractive to pollinators (a “magnet species”; Thomson, 1978), or if both species are equally attractive but pollinator visitation is an accelerating function of density (Feldman *et al.*, 2004)—and the cost of receiving heterospecific pollen is low (*e.g.*, Schemske, 1981). Changes in the assemblages of species flowering together at the local patch scale may therefore alter the patch-selection decisions of pollinators. Over a longer term and larger scale, pronounced

reductions in flowering overlap within guilds of pollinator-sharing plants could mean that those pollinators face resource shortages during particular periods of the flowering season, with unknown consequences for pollinator populations. Long-term studies of plant–insect interactions in a community context—and their consequences for plant populations—would help to answer some of these questions.

A further complication is that climate variation also has a direct impact on pollinator phenology. In a recent synthesis of the evidence that climate change may disrupt plant–pollinator relationships, Hegland *et al.* (2009) point out that, in many cases, both plant and insect phenology appear to be governed by temperature, perhaps making changes in interactions unlikely. However, some studies suggest that plants and pollinators may not respond in parallel to climate change (Kudo *et al.*, 2004; Gordo & Sanz, 2005); and, as our results show, even when members of a community are similarly responsive to environmental cues, shifts in temporal co-occurrence patterns are possible. Population-level consequences of asynchronies between plants and pollinators remain largely untested (Hegland *et al.* 2009).

In conclusion, we have shown that year-to-year variation in snowmelt timing has affected co-flowering patterns in subalpine meadows over the last three decades, influencing the identity and relative abundances of potentially competing plants—despite broad similarities in individual species’ responses to snowmelt date. For *Lathyrus leucanthus*, this has meant flowering concurrently with fewer species overall, and overlapping less with other pollinator-sharing legumes, in early-snowmelt years. We detected no strong temporal trends in community patterns using this dataset, but an increasing frequency of early-snowmelt years with climate warming seems likely to cause long-term change in co-flowering patterns in subalpine communities.

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Chapter 3

Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower

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Abstract

Climate change is causing many plants to flower earlier in spring, exposing them to novel selection pressures, including—potentially—pollinator shortages. Over two years that contrasted in timing of flowering onset, we studied reproductive strategies, pollen limitation, and selection on flowering time in *Mertensia fusiformis*, a self-incompatible, spring-flowering perennial. Plants opened most of their flowers early in the flowering period, especially in 2007, the early year; but selection favoured early-flowering individuals only in 2008. However, resource allocation to early vs. late seed production was flexible: In 2008, but not 2007, early flowers on a plant produced more and heavier seeds. Late flowers were capable of equal seed production if fertilization of early ovules was prevented, suggesting that late flowers serve a bet-hedging function. Evidence for pollen limitation was weak, although there was a tendency for early flowers to be pollen limited in 2007 and for late flowers to be pollen limited in 2008. Poor reproductive success in 2007 was likely attributable less to pollen limitation than to frost damage to flowers. We suggest that plasticity in floral longevity and resource allocation among flowers will make this species resilient to short-term pollinator deficits; whether this will help or hinder future adaptation is unclear.

Introduction

Climate change has given a new urgency to the need to understand both how flowering phenology affects plant reproductive success and the ability of populations to adaptively adjust

flowering time in response to changing conditions (Elzinga *et al.*, 2007). Timing of flowering has long been a popular research topic because it is such an important determinant of fitness in plants (*e.g.*, Rathcke & Lacey, 1985; Pilson, 2000; Sandring *et al.*, 2007): Within a growing season, an individual's flowering period must coincide with favourable climatic conditions, flowering of conspecifics, and availability of abiotic resources and mutualist partners. As global temperatures rise, the windows of climatic suitability, resource availability, and presence of interaction partners will likely all shift—though not necessarily in concert. Climate change has already altered flowering phenology of many species (Parmesan, 2006; Rosenzweig *et al.*, 2007), presumably often because of plastic responses to changing environmental cues (although evolutionary change in flowering schedules has also been documented [Franks *et al.*, 2007, Franks and Weis, 2008]). However, we still know little about the consequences of these changes in flowering phenology for plant populations: For instance, will plastic shifts to earlier flowering lead to reduced out-crossing or seed set, and, if so, will selection begin to favour later-flowering individuals?

In temperate habitats, plants that flower early in spring have always faced particular challenges associated with a harsh and unpredictable environment. Possible benefits of spring flowering include high levels of light and moisture, and low levels of interspecific competition for pollinators. On the other hand, weather can be cold or erratic, and this might limit seed production directly by damaging floral tissue, or indirectly by reducing pollinator activity (Schemske *et al.*, 1978; Kudo *et al.*, 2008). Climate change may exacerbate these problems: For example, early snowmelt in recent years has been associated with increased frost damage to developing flowers of some subalpine plants—and a consequent reduction in recruitment (Inouye, 2008). This occurs because timing of plant development advances in response to earlier melt, but frost frequency does not necessarily decline along with the increase in mean temperatures (Hänninen, 1991; Inouye, 2008). Similarly, early melt and correspondingly early flowering might not be accompanied by early pollinator emergence if insect activity is regulated by different environmental cues (*e.g.*, temperature; Willmer & Stone, 2004). Plants growing in regions with naturally variable spring-time climate may be relatively resilient to these consequences of climate change, but this has been largely untested.

Plants may employ a variety of strategies to cope with an erratic early-season environment in which pollinator visits are infrequent, including self-compatibility or a generalized pollination system (Lloyd, 1992; Waser *et al.*, 1996). A prolonged flowering period, resulting from the production of many flowers or, alternatively, a few long-lived flowers, would increase chances of pollinator visitation (Primack, 1985; Rathcke, 2003; Elzinga *et al.*, 2007). Cold temperatures can increase floral longevity (Yasaka *et al.*, 1998; Vesprini & Pacini, 2005); and although a reduced rate of senescence might simply be a metabolic consequence of lower temperatures, it may also serve an adaptive function by maintaining attractiveness of flowers that are unlikely to have been visited. In many plants, corolla wilting and abscission is also affected by pollen receipt, such that flowers remain attractive for longer if unpollinated (van Doorn, 1997; Ishii & Sakai, 2000; Clark & Husband, 2007). This strategy has the benefit of tailoring both the longevity of individual flowers and, potentially, overall floral display size to variable pollinator availability (Harder & Johnson, 2005).

In plants that produce many flowers in a growing season, the temporal pattern of flower opening (*i.e.*, the flowering schedule) and patterns of resource allocation among flowers can be viewed as strategies for maximizing offspring quantity and quality—though some elements of inflorescence architecture and development are no doubt phylogenetically constrained (Prusinkiewicz *et al.*, 2007). A skewed flowering schedule, in which most flowers on a plant open early and a few open late, might serve to attract and maintain loyal pollinators throughout the flowering period (Thomson, 1980; Makino & Sakai, 2007)—or it might function simply to ensure the earliest possible development for the bulk of a plant's ovules. Typically, reproductive success of a strategy is measured at the scale of whole plants, but studying within-plant variation can be illuminating (Wesselingh, 2007). For example, declining seed set is often observed in later or distal flowers within inflorescences (Stephenson, 1981; Thomson, 1989). So far, most evidence supports two alternative (but not mutually exclusive) hypotheses to explain this observation: The earliest-fertilized ovules may take precedence in within-plant competition for resources (Stephenson, 1981; Medrano *et al.*, 2000; Humphries & Addicott, 2004), or the earliest flowers may have structural advantages such as proximity to nutrient sources or possession of a greater numbers of ovules (Thomson, 1989; Diggle, 1997; Buide, 2008). However, temporal variation in pollinator availability could also generate within-plant variation in seed set. An ability to adjust resource allocation in favour of later flowers if early flowers are unsuccessful would presumably

be beneficial under a changing environmental regime, while a fixed strategy of investment in early flowers would be risky. An ability to adjust flower number and duration of the flowering period would also be advantageous when length of the growing season is unpredictable (Prusinkiewicz *et al.*, 2007).

In this study, we investigated reproductive strategies of a many-flowered perennial, *Mertensia fusiformis* (Boraginaceae), that flowers in early spring in subalpine meadows. We compared the success of these strategies in a year with a relatively early start to the growing season (2007) and a relatively late year (2008). In particular, we tested whether plants are capable of autogamous self-pollination, whether they can adjust floral longevity in response to pollination, and whether they can adaptively reallocate resources to late flowers if early flowers are not pollinated. In light of this information, we asked how pollen limitation varied through the growing season and whether there was selection on timing of flowering. Our objective was to determine whether these early-flowering plants are vulnerable to early-season mismatch with the timing of pollinator activity, particularly in a year of early snowmelt, and whether this affects the pattern of selection on flowering time.

Methods

Study species

Mertensia fusiformis Greene (“alpine”, “spindle-root”, or “dwarf bluebells”) is a long-lived herbaceous perennial that grows abundantly in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL), near Crested Butte, Colorado. It is one of the first species to flower in spring, typically blooming 7–14 days after snowmelt (Inouye *et al.*, 2000). Early in its flowering period, the only other concurrently flowering species in these habitats is *Claytonia lanceolata* Pursh (Portulacaceae). Later in the season, flowering overlaps with *Viola praemorsa* Douglas ex Lindl. (Violaceae; sometimes identified as *V. nuttallii* in previous work at the RMBL), *Delphinium nuttallianum* Pritz. (= *D. nelsonii*; Ranunculaceae), *Lathyrus lanszwertii* Kellogg (= *L. leucanthus*; Fabaceae), *Taraxacum officinale* Weber ex F.H. Wigg (Asteraceae) and other species. Timing of peak flowering in *M. fusiformis* has advanced by approximately five days per decade since 1973, corresponding to a similar advance in snowmelt date in the same time-period (Miller-Rushing & Inouye, 2009).

Like other Boraginaceae, *M. fusiformis* has a determinate cymose inflorescence, in this case composed of numerous densely clustered campanulate flowers. Flowers are ~1 cm long, changing from pink at the bud stage to blue as they open. We did not measure temporal variation in stigma receptivity or pollen viability, but anthers typically open within 24 hours of corolla-opening, and there are no obvious physical changes in the stigma over the course of the flowering period. In *Mertensia ciliata*, stigmas remain receptive even after corollas and stamens have been shed (Pelton, 1961; Geber, 1985); this may also be the case in *M. fusiformis*. The plant reaches a maximum height of approximately 15 cm, and later-opening flowers are borne lower on the plant and in smaller clusters than earlier flowers. The most conspicuous flower-visitors are nectar-collecting bumble bee queens, primarily *Bombus bifarius* at our study sites; however, the species is also visited by solitary bees, including *Osmia* spp. Fruits are one-seeded nutlets that have no specialized dispersal mechanisms other than an elaiosome that is attractive to ants (Turnbull *et al.*, 1983). Herbivore damage at our study sites was rare, but stems were occasionally grazed, presumably by deer. Many plants showed signs of water stress during the fruiting period, and vegetative growth ceases well before the start of autumn frosts, likely in response to summer drought.

Study sites

We established two study sites 2.4 km apart, near the RMBL. The first (“Avery”; 38°58.27'N, 106°59.73'W) was a 40 × 60 m area of meadow with numerous *M. fusiformis* that included dry, rocky sections and more level, wetter areas. At this site, on 9 May 2007, we selected and marked with red pin flags 195 plants that were not yet in flower, along with the only six plants in the study area that were already flowering (five of these apparently having begun to flower that day). Because we were particularly interested in early-flowering plants, these were over-represented in our sample; estimates of population flowering distributions were therefore not calculated for this site. At the end of the season, all plants were permanently marked with metal tags and their locations mapped so they could be recovered in 2008. However, only a subset of these plants were relocated in 2008, so an additional 100 plants were newly flagged in the second year.

The second site (“South Gothic”; 38°57.13'N, 106°58.88'W), studied in 2008 only, was limited to a 20 × 50 m area of more uniformly dry, rocky ground. Snow melted early here in comparison both with the surrounding lower-lying areas and the Avery site. Two hundred plants were

selected on 23 May 2008, before they flowered, by placing three 30 cm × ~50 m transects and flagging all plants with buds that lay within the transect. We also flagged an additional 20 plants that appeared likely to flower soon to ensure a larger sample of early-flowering plants; these non-randomly selected plants were not included in measurements of population-level flowering patterns.

Field methods, 2007

Plants were monitored daily, except on three days of especially snowy weather. All newly flowering plants were noted and the number of open flowers per plant on each day was counted for a subset of plants that included at least five newly flowering plants per day, when possible.

Pollen limitation

For the first 25 plants to flower (excluding the six already in bloom), all flowers to open over the first five days of the flowering period were marked on sepals with a black permanent marker to indicate flower rank within that plant (*i.e.*, flowers opening on the first day were “rank 1”, second-day flowers were “rank 2”, etc.). After the fifth day of flowering, all flowers on a plant were grouped as “rank 6”. Of these 25 plants, 12 were randomly assigned to a pollen-supplementation treatment. Open flowers on these plants received outcross pollen on each day the plants were monitored. For six of these plants, all flowers on the plant were pollinated; but the other six plants were prohibitively large (plants can produce as many as 900 flowers), so that only a subset of flowers (those on one or two stems) received supplemental pollen (see *Data analysis*). For pollinations, we collected dehiscing anthers from plants growing outside the study plot and brushed these across the stigmas of the selected flowers—though within-population variation in style length meant that stigmas were often concealed beneath the ring of anthers, making precise placement of outcross pollen difficult. Multiple pollen donors were used each day, and all selected flowers were pollinated on each day the flowers were open, so that each outcrossed flower was likely to receive pollen from multiple pollen donors over the course of its lifespan.

Mating system and floral longevity

To test for self-compatibility and the effect of pollen receipt on floral longevity, we selected 37 additional plants and covered these with 1 mm mesh bags prior to flowering to exclude pollinators. Bagged plants were assigned to three treatments: control (unmanipulated), self-pollination, and cross-pollination. On pollinated plants, in the first 5–6 days of flowering, newly opened flowers were marked as described above to indicate flower rank. Flowers were considered open when the corolla had opened sufficiently to make all anthers fully visible; typically this coincided with the flower turning from pink to blue (anther dehiscence was not used as a criterion, but anthers usually dehisced later the same day). Approximately half of the monitored flowers received a black mark on the pedicel and were hand-pollinated; the remainder were handled for marking but otherwise unmanipulated. Outcross-pollination was conducted as described above for unbagged plants. Self-pollination was accomplished by using forceps to transfer pollen from the anthers of the same flower or other flowers of the same plant to stigmas of the selected flowers. We noted the day on which each flower shed its corolla. Bags were removed after plants had finished flowering.

Seed counting

We monitored fruit set in 76 unmanipulated plants that spanned the range of first flowering dates (approximately five per first flowering day between 9 and 26 May, plus the single plant that was already in flower, assigned an estimated first flowering date of 8 May) as well as in the 12 hand-pollinated and 37 bagged plants. Nutlets were considered to have successfully matured once they dropped from the plant; this allowed us to determine seed set by examining abscission scars in cases where nutlets detached while we were absent. Abscission scars were easily distinguished from undeveloped ovules and were therefore a reliable indicator of the number of matured fruits. Because *Mertensia* spp. have four ovules per flower, percent seed set could be calculated as $(100\% \times \text{number of mature nutlets}) \div (4 \times \text{number of flowers})$. This is likely an overestimate of the number (or proportion) of viable seeds, however, because some of the nutlets that did detach were soft. We did not expect differences in seed mass or viability between fruits that fell naturally from plants and those we collected manually, because whether a nutlet detached on its own seemed to depend only on the orientation of the flower pedicel and whether the infructescence was physically disturbed. However, in 2009, we tested this assumption explicitly

by attaching a vial around the inflorescences of 12 plants just before fruit set to collect any abscised nutlets. Vials were monitored for seeds approximately every two days. At five-day intervals (the average frequency at which seeds were collected in 2007 and 2008) we also collected, by hand, mature nutlets that had not dropped into the seed traps. In total, we collected 216 nutlets from 12 plants by hand and 146 nutlets from 11 plants in the seed traps. We found no difference between seeds collected manually or passively, either in terms of mean seed mass (0.87 ± 0.06 mg [mean $\pm$ 1 s.e.] for manually collected seeds vs. 0.86 ± 0.04 mg for seeds in traps; paired t -test, $t_{10} = 0.40$, $P = 0.70$) or proportion of seeds that could be crushed with forceps (0.070 ± 0.027 for manually collected seeds vs. 0.104 ± 0.048 for seeds in traps; Wilcoxon signed-rank test, $P = 0.95$, $V = 21.5$, $N = 11$).

Field methods, 2008

We visited the Avery site four times during snowmelt in 2008 to map the boundaries of the retreating snow. This allowed us to assign approximate snowmelt dates to individual plants, based on their mapped locations (see *Data analysis*). When flowering began, plants at both sites were visited every second day (except for one day missed because of snow), instead of every day as in 2007. All open flowers were counted on each census day. We monitored fruit set on a subset of approximately 10 unmanipulated plants per first flowering date per site, for a total of 84 or 85 plants per site; however, at the South Gothic site, one plant was grazed, and data from another two were omitted following an error in data entry, leaving us with a sample size of 81. As many seeds as possible were collected as they matured for later weighing. Thus, in 2008, we had mean seed mass (per seed) and seed set as response variables.

Pollen limitation

At each site, 40 of the first plants to flower were alternately assigned to “pollinated” or “control” treatments. Newly opening flowers were marked as described above, except that “rank 1” now included flowers opening on the first two days of the flowering period, “rank 2” flowers opened on days 3 and 4, and so on. Each day, a subset (up to half) of the new flowers on “pollinated” plants received supplemental pollen; the other flowers on these plants, and the “control” plants, were unmanipulated. This allowed us to compare seed set between control and hand-pollinated flowers on the same plant, and between unmanipulated flowers on control and (incompletely)

hand-pollinated plants. Hand-pollinated flowers received an additional black mark on the pedicel but were otherwise indistinguishable from unmanipulated flowers. Because flowers are tightly clustered within the inflorescence, pedicels are typically concealed, and these marks are unlikely to have influenced pollinator behaviour. Pollination techniques were the same as described for 2007. Seeds were collected to obtain mean seed mass for flowers of each rank and treatment on each plant.

Resource allocation between early and late flowers

To test for inherent differences in fecundity and trade-offs in allocation between early and late flowers, we conducted a second bagging experiment at the Avery site in 2008. Before plants began to flower, we bagged 12 sets of three similar-sized plants that each had at least 30 buds. On all plants, we marked the first 10 and the last 10 flowers to open. Within each set of three, plants were arbitrarily assigned to one of three treatments: early pollination (first 10 flowers received outcross pollen), late pollination (last 10 flowers pollinated), or both early and late pollination. Seeds were weighed to obtain an average seed mass for each cohort (early or late) of each plant.

Data analysis

Analyses were conducted in R (R Development Core Team, 2007).

Flowering patterns

In comparing flowering patterns between years, we calculated flowering duration for individual plants as *last flowering day* – *first flowering day* + 1 in 2007, and *last flowering day* – *first flowering day* + 2 in 2008 (we assume that sampling only every second day in the latter year would cause us to underestimate flowering duration by one day, on average). Skewness in flowering schedules of individual plants (g) was calculated as the sample skewness using the `npde` package in R, while population skewness (G) was calculated and tested using the `moments` package.

We used multiple linear regression to find the best predictor of flowering date among permanently tagged plants at the Avery site, including 2008 snowmelt date (at the location of the individual plant), plant size, and the date on which the plant flowered in 2007 as explanatory

variables. We had mapped snowmelt boundaries at 2- to 4-day intervals (on 26 and 29 May, and 2 and 4 June 2008), giving us lower temporal resolution for snowmelt dates than for flowering dates. Therefore, to avoid underestimating the explanatory power of snowmelt date, we inferred an additional snow contour for 31 May, positioned mid-way between the observed contours for 29 May and 2 June. Individual plants were assigned one of the five possible snowmelt dates according to their locations relative to these contours. The maximum number of open flowers on a single day (log-transformed) was used as an estimate of plant size, as it was strongly correlated with the total number of flowers produced by a plant (square-root-transformed; Pearson's $r > 0.85$, $P < 0.0001$ in both years), but was more easily obtained. Flowering date in 2007 was rank-transformed to reduce the influence of one late-flowering outlier. We selected the best model as the one having the lowest AIC (Akaike Information Criterion) value, and we used likelihood-ratio comparisons of nested models to evaluate the significance of additional terms. Despite moderate correlations among predictor variables, all variance inflation factors (VIFs) were less than 1.4, indicating that our parameter estimates are robust (Quinn & Keough, 2002).

Floral longevity

Differences in floral longevity between pollinated and unpollinated flowers on the same plant were evaluated with paired t -tests (conducted separately for self- and cross-pollinated plants).

Pollen limitation

Because of the large number of zero values and unequal sample sizes, these data did not lend themselves to parametric statistical analyses. We therefore tested for treatment effects on flowers of each rank separately, using non-parametric tests, and applied sequential Bonferroni correction to account for the multiple testing. In 2007, we used Kruskal–Wallis tests to compare seed set of all flowers of a given rank on pollen-supplemented and unmanipulated plants, even though some of the former plants included flowers that did not receive supplemental pollination. In 2008, we were able to test for an effect of pollen supplementation within manipulated plants: For flowers of a given rank, we tested whether the differences in seed set or seed mass between hand-pollinated and open-pollinated flowers differed significantly from zero, using a Wilcoxon signed-rank test. We also compared seed set and seed mass of open-pollinated flowers on manipulated plants to those of unmanipulated plants, to ensure that enhanced seed set of

supplemented flowers had not caused a corresponding reduction in seed set of control flowers on those plants (Zimmerman & Pyke, 1988). To better allow comparison between years, we re-analysed 2007 data after lumping flowers into ranks that corresponded with three of those used in 2008 (rank 1: flowers from days 1 and 2, rank 2: days 3 and 4, rank 3: day 5 and beyond). We tested for trends in seed set and seed mass with increasing flower rank by calculating rank correlations (Spearman's ρ) for each plant and then testing whether the set of correlation coefficients for each treatment deviated from a mean of zero using a Wilcoxon signed-rank test.

Resource allocation

Mean seed masses were normally distributed, so differences between treatments were tested by one-way ANOVA. Percent seed set values were not normally distributed, even after arcsine-transformation, so a Kruskal–Wallis test was used instead.

Selection on flowering schedules

We used number of seeds per plant and total seed mass (the product of number of seeds per plant and mean seed mass, measured in 2008 only), proxies for fitness via female function, to estimate the strength of phenotypic selection on flowering time and skewness of the flowering schedule. Lifetime fitness measures were beyond the scope of our two-year study; however, these shorter-term measures of selection allowed us to evaluate whether selection differed between years and between sites. Linear selection differentials were calculated as the covariance between relative fitness (an individual's seed production divided by the population mean in that year) and the trait; significance was tested by Pearson correlations. Linear selection gradients were obtained from multiple regressions of relative fitness on flowering date, flower number, and skewness of a plant's flowering schedule. The partial regression coefficient for a trait is a measure of the strength of selection acting directly on that trait, instead of indirectly via an effect on the other measured traits (Lande & Arnold, 1983). We checked that all VIFs were below 2, suggesting that our parameter estimates were not affected by problems of multicollinearity. Selection gradients (β') are reported in s.d. units. Given our relatively small sample sizes and the absence of obvious non-linearity in the relationships between traits and fitness, we did not attempt to measure non-linear selection. For significance testing, number of seeds per plant and total seed mass per plant were 4th-root-transformed, and flower number was square-root transformed.

To determine the extent to which estimates of selection via female function (seed set) also reflect selection via the male component of fitness (seed siring), we estimated the level of assortative mating by flowering date based on unmanipulated plants at the South Gothic site (2008), following the method of Weis and Kossler (2004). For all flowers open on a given maternal parent on a given day, we estimated the mean first flowering date of potential pollen donor plants by weighting first flowering dates of all other censused plants in the population by the number of flowers they had open on that day. The values for each day were weighted by the proportion of open flowers on the maternal parent on that day to obtain the mean first flowering day of pollen donors for that plant. The correlation in first flowering dates between potential pollen donors and recipients (ρ) is a measure of assortative mating. Spatial proximity of prospective mates was not taken into account. Similarly, because we had no evidence of dichogamy, it was not considered in calculations of assortative mating. However, not all flowers on a plant are equally likely to set seed; temporal variation in fecundity would change the distribution of potential sires from that predicted based on the flowering schedule alone (Weis & Kossler, 2004). We therefore calculated a second estimate of ρ , weighting flowers by their expected contribution to a plant's seed production (estimated from the mean seed set observed for flowers of different ranks on the control plants at this site).

Results

Population-level flowering patterns

Weather patterns in the two years of our study differed dramatically: Of the last 34 years (1975–2008), 2007 was the year with the fifth-earliest date of spring snowmelt at the RMBL, while 2008 ranked 27th (RMBL, 2009). This caused permanently tagged and relocated plants ($N = 67$) at the Avery site to flower on average 27 days later in 2008 than they did in 2007. Larger plants (those with a larger peak floral display) tended to flower earlier, but the strength of this relationship varied between sites and years, being strongest at the Avery site in 2007 (the site-year with the earliest start to the flowering season; Pearson's $r = -0.57$, $N = 76$, $P < 0.0001$), weaker at the South Gothic site ($r = -0.29$, $N = 160$, $P = 0.0002$), and non-significant at Avery in 2008 (the latest site-year; $r = -0.12$, $N = 133$, $P = 0.17$). This pattern was unchanged if total number of flowers was used as the response variable instead of maximum floral display.

Nevertheless, date of first flowering was highly correlated between years for individual plants (Spearman's $\rho = 0.62$, $N = 67$, $P < 0.0001$). Peak flowering displays of tagged plants were larger in 2008 (paired t -test, $t_{34} = 2.89$, $P = 0.0067$; Fig. 3.1), but there was no detectable difference in total flower number per plant between years (paired t -test, $t_{24} = 0.84$, $P = 0.41$), suggesting that the increased display size was driven at least in part by the compressed flowering season in the later year. Indeed, duration of flowering for individual plants was, on average, 9.5 days shorter in 2008 (paired t -test, $t_{34} = 10.2$, $P < 0.0001$; Fig. 3.1).

Snowmelt date at the location of individual plants was a good predictor of flowering date in 2008, but better models also included the date on which a plant flowered in 2007, regardless of whether plant size was also included in the model (Table 3.1). That the inclusion of 2007 flowering date significantly improved model fit suggests that plant attributes other than size and position relative to snowmelt gradients contributed to determining flowering time.

The randomly selected plants at the South Gothic site in 2008 showed a positively skewed flowering distribution, with most flowers in the population opening early in the flowering period ($G = 0.229$; D'Agostino skewness test, $P < 0.0001$). This skew resulted from a weakly positively skewed distribution of first flowering dates ($G = 0.116$, $P = 0.66$) in combination with skewed flowering schedules of individual plants (pollinated plants excluded; mean within-plant $g = 0.123$, one-sample t -test against a hypothesized mean of 0, $t_{159} = 4.63$, $P < 0.0001$; Fig. 3.1). Individual plants also showed positively skewed flowering schedules at the Avery site in both years (mean within-plant $g = 0.305$ in 2007, $g = 0.168$ in 2008, both $P < 0.0001$; Fig. 3.1). Skewness was moderately consistent between years for individual plants ($r = 0.476$, $N = 35$, $P = 0.0038$), but most plants had more positively skewed flowering schedules in 2007 than in 2008 (paired t -test, $t_{34} = 3.84$, $P = 0.0005$; Fig. 3.1).

Mating system and floral longevity

Unmanipulated flowers on bagged plants (Avery site, 2007) set a negligible number of seeds, and hand-self-pollinated flowers set none (Table 3.2), indicating self-incompatibility. Flowers that were outcross-pollinated by hand successfully set seed, and shed corollas, on average, two days earlier than control flowers on the same plant (Table 3.2; paired t -test, $t_{10} = 6.2$, $P <$

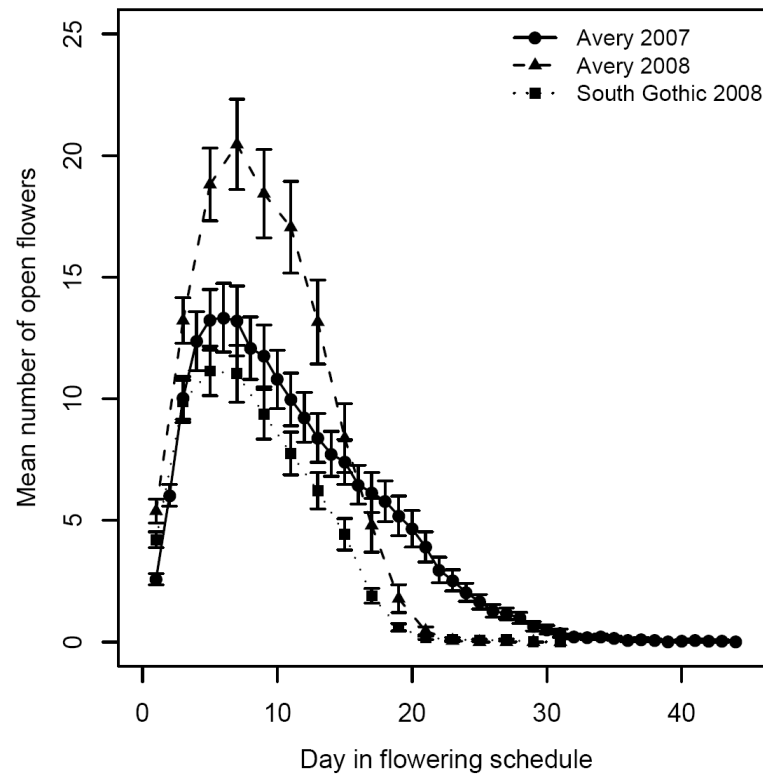


Figure 3.1. Average flowering schedules, showing mean number of open flowers per plant on each day of the flowering period (± 1 s.e.); note that the first flowering day for each plant is treated as day 1 of the flowering schedule regardless of the calendar date of first flowering. For Avery 2007, $N = 75$; for Avery 2008, $N = 133$; for South Gothic, $N = 161$.

Table 3.1. Models predicting flowering time for plants at Avery site in 2008 ($N = 65$), based on snowmelt date in 2008, plant size (as $\ln[\text{maximum floral display size}]$) in 2008, and 2007 flowering date (rank-transformed). Likelihood-ratio χ^2 statistics (on 1 df) and P -values test the significance of the last model term relative to the next-simplest nested model. Interaction terms did not significantly improve model fits. The signs of estimated effects are given in parentheses beside each model term.

Predictors (Sign of estimated effect)	R^2	AIC	χ^2	P
Size (–)	0.00	385	0.2	0.665
2007 flowering date (+)	0.38	353	32.1	<0.0001
Snowmelt (+)	0.66	314	70.9	<0.0001
Snowmelt (+), size (–)	0.68	311	5.7	0.017
Snowmelt (+), size (–), 2007 flowering date (+)	0.74	297	15.2	0.0001
Snowmelt (+), 2007 flowering date (+)	0.73	299	16.9	<0.0001
Snowmelt (+), 2007 flowering date (+), size (–)	0.74	297	4.1	0.043

Note: AIC = Akaike Information Criterion

Table 3.2. Floral longevity and fruit and seed set of bagged plants (2007). Plants were assigned to three treatments (control, self-pollinated, or outcross-pollinated). Within pollinated plants, flowers opening on the first 5–6 d of flowering were assigned either to hand-pollination or control treatments. Floral longevity was not monitored in control plants. Values are means $\pm$ 1 s.e., with sample sizes (numbers of plants) in parentheses.

Variable	Control	Self-pollinated		Outcross-pollinated	
	Control	Pollinated	Control	Pollinated	Control
Longevity (d)	—	3.3 $\pm$ 0.3 (10)	4.8 $\pm$ 0.4 (10)	3.2 $\pm$ 0.2 (11)	5.2 $\pm$ 0.5 (11)
Fruit set (%)	1.0 $\pm$ 0.6 (13)	0.0 $\pm$ 0 (11)	0.10 $\pm$ 0.07 (11)	44.2 $\pm$ 5.3 (12)	0.5 $\pm$ 0.3 (12)
Seed set (%)	0.3 $\pm$ 0.2 (13)	0.0 $\pm$ 0 (11)	0.03 $\pm$ 0.02 (11)	27.3 $\pm$ 3.6 (12)	0.3 $\pm$ 0.2 (12)

0.0001). Interestingly, despite its inability to fertilize ovules, self-pollen also reduced floral longevity (by 1.5 days; paired t -test, $t_9 = 4.6$, $P = 0.0012$). This reduction does not differ significantly from that produced by outcross pollen (t -test on differences, $t_{19} = 1.0$, $P = 0.32$).

Pollen limitation

Among the early cohort of plants that were used to test pollen limitation at the Avery site in 2007, seed set was low overall and did not differ significantly between treatments at the level of whole plants ($6.0 \pm 1.0\%$ [mean $\pm$ 1 s.e.] for control plants, $10.4 \pm 2.4\%$ for pollinated plants; Kruskal–Wallis $\chi^2 = 0.855$, $df = 1$, $N_1 = 13$, $N_2 = 12$, $P = 0.36$). The earliest flowers showed the greatest difference in seed set between control and pollinated treatments (Fig. 3.2). This difference was not significant, particularly after Bonferroni correction (Kruskal–Wallis $\chi^2 = 3.19$, $df = 1$, $N_1 = 13$, $N_2 = 12$, $P = 0.074$, $\alpha = 0.05/6 = 0.0083$), but the trend for pollen limitation in the earliest flowers remained when flowers from the first two days were combined for analysis, for comparison with 2008 results ($6.2 \pm 2.0\%$ seed set in unmanipulated vs. $13.4 \pm 3.0\%$ in pollinated plants, Kruskal–Wallis $\chi^2 = 3.00$, $df = 1$, $N_1 = 13$, $N_2 = 12$, $P = 0.083$). In control plants, seed set increased with flower rank (mean $\rho = 0.21$, Wilcoxon signed-rank test on correlation coefficients, $V = 64.5$, $N = 12$, $P = 0.050$); pollinated plants, in contrast, showed no temporal trend in seed set (mean $\rho = -0.11$, $V = 24$, $N = 11$, $P = 0.45$).

In 2008, seed set among experimental plants was higher at both sites than it was at Avery in 2007 ($22.7 \pm 2.9\%$ for pollinated and $20.3 \pm 2.3\%$ for control plants at Avery; $35.3 \pm 3.1\%$ for pollinated and $27.7 \pm 3.6\%$ for control plants at South Gothic). Overall, the differences between pollen supplemented and unmanipulated plants were not significant (Kruskal–Wallis $\chi^2 \leq 3$, $df = 1$, $0.20 > P > 0.05$ at both sites); however, the strength of the treatment effect appeared to vary with flower rank (Fig. 3.2). Among the latest-opening (rank 6) flowers at both sites, hand-pollinated flowers set more seed than their unmanipulated counterparts, although this was not significant after Bonferroni correction (Wilcoxon signed-rank tests, $V = 78$, $P = 0.025$ at Avery, and $V = 72$, $P = 0.011$ at South Gothic; $\alpha = 0.05/6 = 0.0083$). Increased seed set by pollinated flowers was not associated with a decline in seed set of unmanipulated flowers, compared to flowers of the same rank on control plants (Kruskal–Wallis $\chi^2 < 2.5$, $df = 1$, $P > 0.1$ for rank 6 flowers at both sites). At both sites, seed set of unmanipulated flowers declined strongly with increasing flower rank (Fig. 3.2; mean rank correlations = -0.41 and -0.67 at Avery and South

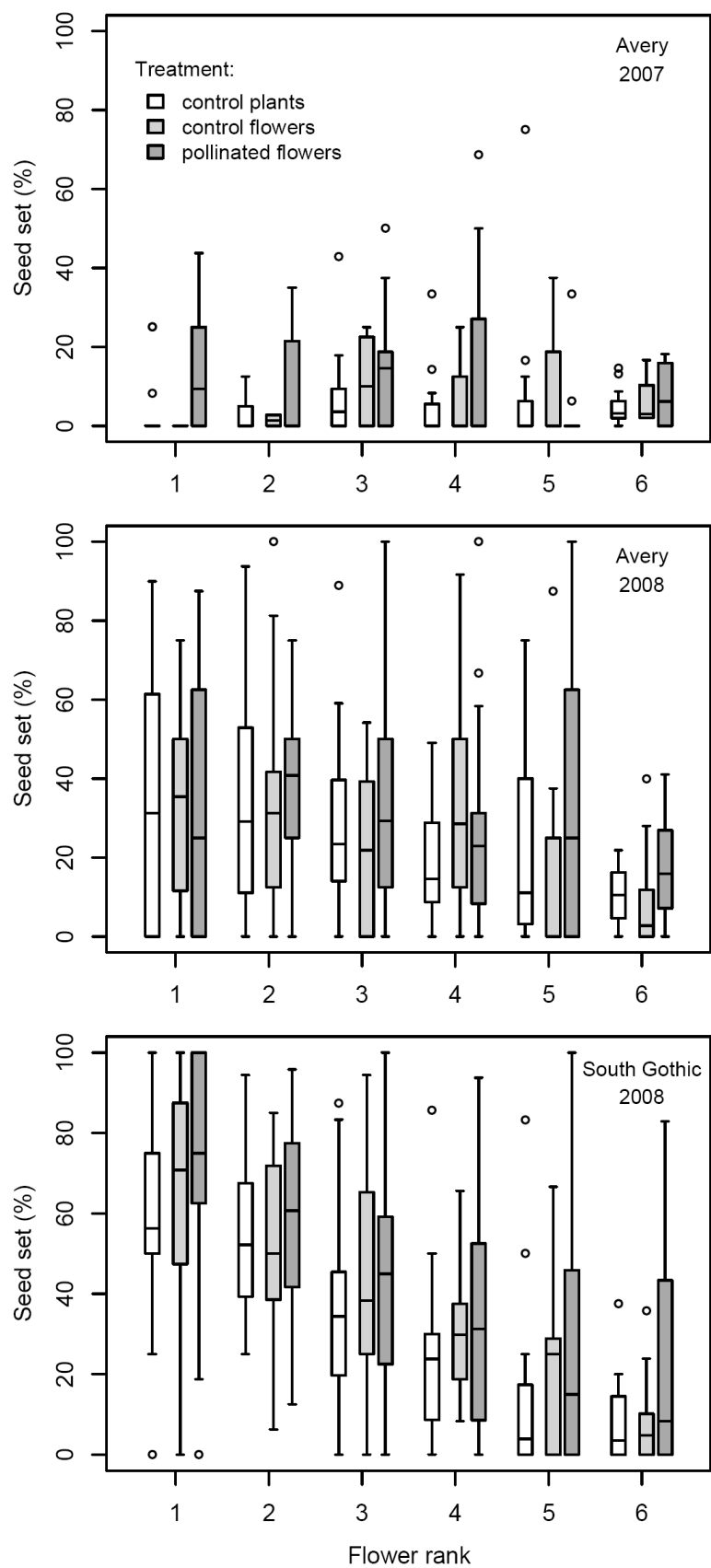


Figure 3.2. (*opposite page*) Tukey box-plots showing percent seed set of pollinated and control plants vs. flower rank at each site-year. Flowers on pollinated plants either received supplemental pollen (dark gray bars) or did not (light gray bars); no flowers on control plants received supplemental pollen (open bars). Flower rank in 2007 refers to the day in the plant's flowering schedule on which the flower opened; in 2008, ranks are two-day periods instead of single days (see *Field methods 2008, Pollen limitation*). Boxes show the median and interquartile range.

Gothic respectively; Wilcoxon $P < 0.01$ in both cases). This decline was alleviated somewhat in pollen-supplemented flowers at Avery (mean $\rho = -0.22$, Wilcoxon $V = 36$, $N = 17$, $P = 0.058$), but not at South Gothic (mean $\rho = -0.62$, Wilcoxon $V = 12$, $N = 19$, $P = 0.0009$). Mean seed mass (per seed) followed a similar decline with increasing flower rank as was observed for seed set in 2008 (the only year in which seeds were weighed). This decline was consistent across the two sites and both flower treatments (mean within-plant $\rho < -0.4$, all Wilcoxon $P < 0.02$, Fig. 3.3). There was no effect of pollination treatment on mean seed mass, regardless of flower rank (Wilcoxon signed-rank tests, all $P > 0.1$).

Resource allocation

When pollinators were excluded and only the 10 earliest and/or 10 latest flowers were pollinated (2008 bagging experiment), there was no difference in seed set or mean seed mass between early and late flowers (Table 3.3), indicating that the declines in seed set and seed mass observed in open-pollinated plants were not due to structural differences between early and late flowers. Furthermore, pollination of the 10 earliest flowers did not reduce seed size or seed production in the latest flowers (Table 3.3), suggesting there was not a strong trade-off in resource allocation between early and late flowers. Mean seed mass of “late” (rank 6), pollinated flowers on unbagged plants at the same site was lower than that of late flowers on the late-treatment bagged plants (planned comparison; Welch's $t = 2.33$, $df = 15.6$, $P = 0.034$), while there was no such difference between early (or rank 2) flowers of bagged and unbagged plants (Welch's $t = 1.14$, $df = 22.1$, $P = 0.27$). This may indicate that the higher pollination levels achieved by unbagged, pollen-supplemented plants reduced seed mass of later flowers. However, in terms of percent seed set, there were no significant differences between the four treatment-cohort combinations (Kruskal-Wallis $\chi^2 = 0.043$, $df = 3$, $P = 0.998$) or between flowers of bagged and unbagged plants (Kruskal-Wallis $\chi^2 < 2$, $df = 1$, $P > 0.1$ for both early and late flowers).

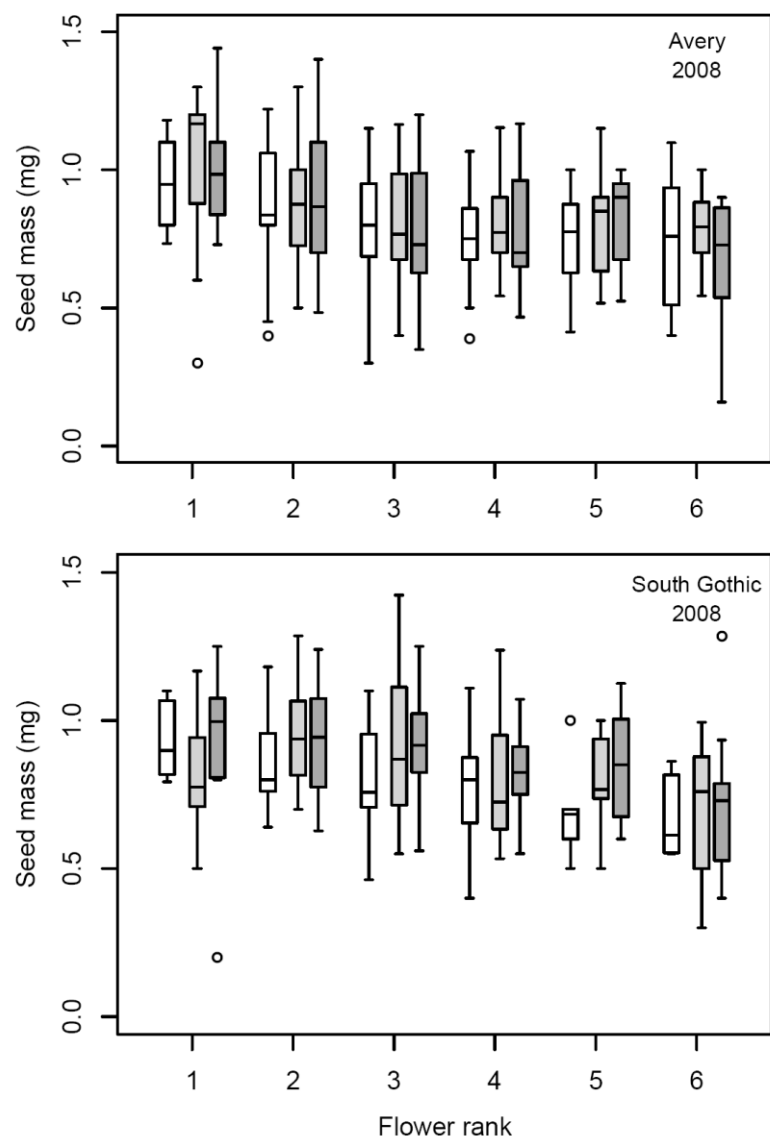


Figure 3.3. Seed mass of pollinated and control plants vs. flower rank at each site-year. Shading as in Fig. 3.2.

Table 3.3. Seed mass and seed set for plants at the Avery site in 2008. Only a subset of flowers on bagged plants (the 10 earliest, 10 latest, or both) were pollinated by hand. Unbagged plants were open to insect pollination; data presented here are for flowers that also received supplemental outcross pollen. Values are means $\pm$ 1 s.e. $N = 12$ bagged plants per pollination treatment and 18 unbagged plants. Superscript letters denote values that were significantly different by Tukey’s HSD ($P < 0.05$). See text for details.

Variable	Bagged plants				Unbagged plants	
	Early pollination	Both early and late pollination		Late pollination	Hand-pollination	
	Early cohort	Early cohort	Late cohort	Late cohort	Early cohort	Late cohort
Mean seed mass (mg)	1.01 ± 0.06^a	1.02 ± 0.08^a	1.00 ± 0.09^a	0.93 ± 0.10^{ab}	0.899 ± 0.08^{ab}	0.667 ± 0.06^{bc}
Seed set (%)	30.2 ± 7.2^a	27.7 ± 5.7^a	31.3 ± 8.4^a	28.7 ± 6.9^a	35.7 ± 5.5^a	16.8 ± 3.2^a

Table 3.4. Estimates of standardized linear selection differentials (s') and gradients (β'), $\pm$ s.e. Flower number was square-root transformed for analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

Character	Avery 2007 ($N = 76$)		Avery 2008 ($N = 85$)		South Gothic 2008 ($N = 81$)	
	s'	β'	s'	β'	s'	β'
Flowering date	-0.25*	0.13 ± 0.12	-0.042	0.078 ± 0.073	-0.51***	$-0.19 \pm 0.071^{**}$
Total no. of flowers	0.71***	$0.78 \pm 0.12^{***}$	0.99***	$1.02 \pm 0.072^{***}$	0.848***	$0.775 \pm 0.070^{***}$
Skewness	-0.087	-0.024 ± 0.10	-0.11	0.070 ± 0.073	-0.019	0.0023 ± 0.063

Phenotypic selection on flowering schedules

Plants that began flowering later in the season generally produced fewer seeds; this resulted in a significant selection differential for flowering time at Avery in 2007 ($s = -1.1$ days) and at South Gothic in 2008 ($s = -1.6$ d), but not at Avery in 2008 (Table 3.4, Fig. 3.4). However, much of this effect was due to indirect selection, acting through flower production; after accounting for number of flowers, direct selection for earlier flowering was significant only at South Gothic (Table 3.4). Considering seed mass (measured in 2008 only) did not qualitatively change this result; direct selection via total seed mass also favoured early plants only at South Gothic ($\beta' = -0.15$, $P = 0.030$). There was no detectable selection on skewness of a plant's flowering schedule (Table 3.4).

At the South Gothic site, most plants showed at least partial flowering overlap with all other plants in the sample because of the extended flowering duration of individual plants and the relative topographical homogeneity of the sampled area. Nevertheless, the correlation in first flowering dates between potential mates, inferred from the flowering schedules of all individuals in the sample, was high ($\rho = 0.714$, $N = 166$) owing to the positive skew in most flowering schedules (*i.e.*, an individual plant produced most of its flowers shortly after its first flowering day). This estimate was increased slightly by incorporating the observed decline in seed set in later flowers ($\rho = 0.741$). Thus, selection on first flowering date, when it occurs, would be manifested through both male and female components of fitness.

Discussion

Determinants of flowering phenology in *M. fusiformis*

Timing of snowmelt and (at least at Avery in 2007 and South Gothic in 2008) plant size had a strong influence on date of first flowering. After accounting for snowmelt date and size, which is likely a function of age as well as genetic and environmental factors, individual plants differed predictably in flowering time. Many studies have demonstrated a genetic basis to flowering phenology (Mazer & LeBuhn, 1999; Franks *et al.*, 2007; Van Dijk & Hautekèete, 2007; Wilczek *et al.*, 2009), and some of the observed individual differences in flowering time in our plants may reflect heritable variation. Unmeasured environmental gradients may also be acting; for instance,

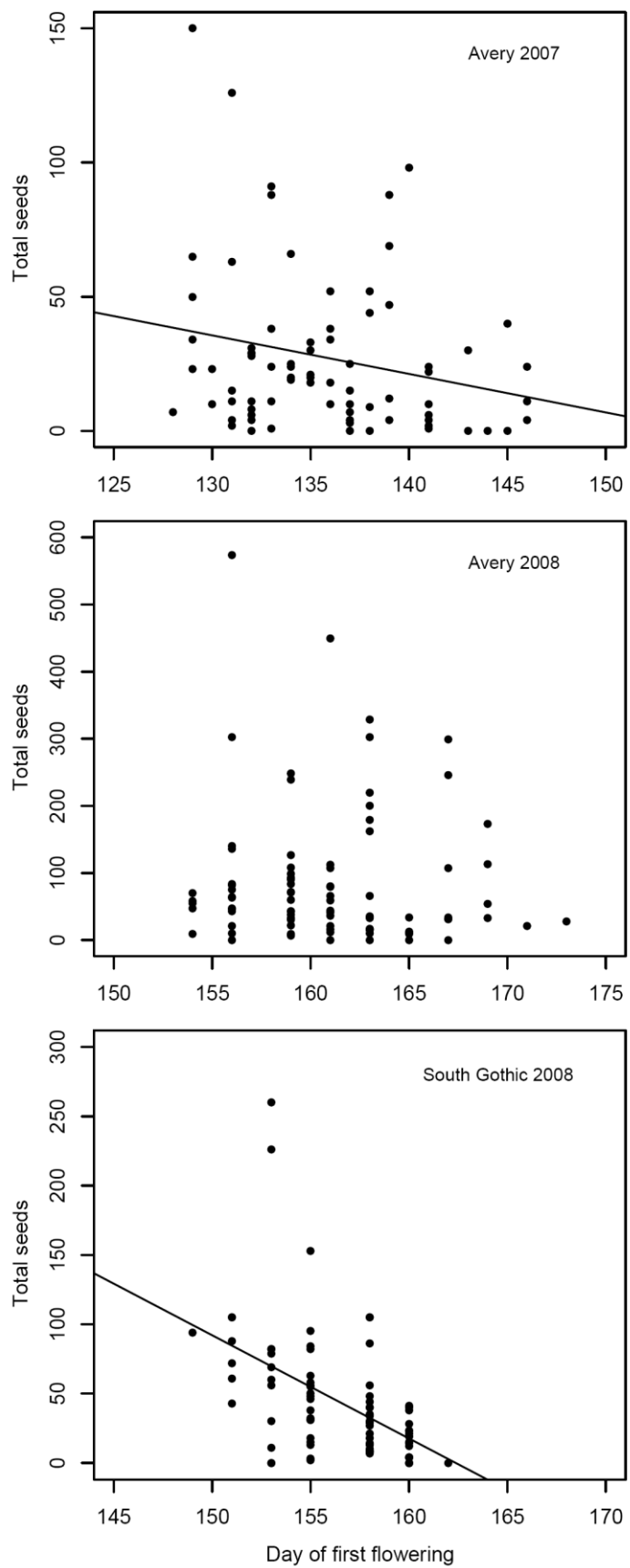


Figure 3.4. (*opposite page*) Total seed production vs. day of year of first flowering for unmanipulated plants (150 = May 30 in 2007, or May 29 in 2008, a leap year). For Avery 2007, $N = 76$; Avery 2008, $N = 85$; South Gothic, $N = 81$. Regression lines are shown only if significant selection differentials were detected. Note variation in y-axis scale.

our estimates of snowmelt date were coarse, and we lack data on small-scale heterogeneity in air temperatures. However, predictability can be viewed as setting an upper limit for heritability.

At our study sites, the shorter 2008 growing season led to shorter, but more intense, flowering periods for individual plants. The compressed season may also explain the lack of a detectable relationship between plant size and date of first flowering at the (later) Avery site. The longer flowering season of 2007 produced more positively skewed flowering schedules; that is, individual plants had longer “tails” of late-opening flowers. It is unlikely that these late flowers represent a plastic response to low seed set in early flowers, as total flower number did not differ significantly between years for individual plants. Because the flowering peaks of individual plants—not only the first flowering dates—shift earlier in early-snowmelt years, most of a plant’s flowers are exposed to early season conditions, although the tail of late flowers provides some insurance against reproductive failure of early flowers. If this were a general pattern, it could also explain why first flowering dates can be an adequate predictor of peak flowering dates in these communities, in spite of fluctuations in population size (Miller-Rushing *et al.*, 2008).

Because timing and duration of flowering in *M. fusiformis* are highly responsive to variation in snowmelt date (this study; Inouye *et al.*, 2000; Dunne *et al.*, 2003), plants can experience dramatically different conditions during the flowering period, depending on whether snow melts early or late. To some extent, this was captured in the two years of our study, which differed markedly in the timing of onset of the growing season. However, *M. fusiformis* possesses traits that, in combination with a perennial habit, confer some resilience to year-to-year fluctuations in climate and synchrony with pollinators, thereby limiting the strength of selection on flowering time. These include extended longevity for unpollinated flowers, and production of many later flowers that probably serve an “insurance” or bet-hedging function. Below, we elaborate on the benefits and limitations of these strategies.

Determinants of seed set

Overall, seed set was lower in 2007 than in 2008. Numerous factors might be responsible, but more severe frost damage in 2007 was undoubtedly important. Two hard frosts in May 2007 damaged many of the flowers at the study site (one frost event, occurring shortly after the peak of flowering, reduced the number of open flowers by ~40%). More frosts occurring after the start of the growing season are a projected consequence of climate warming (Hänninen, 1991), and long-term data from the RMBL suggest that they are an increasingly important problem for frost-sensitive species of subalpine wildflowers (Inouye, 2008). Despite this potential cost to early flowering, in the plants we studied, seed production was typically greatest in early-flowering individuals. This pattern arose because the frosts were not confined to the beginning of the growing season, and because early plants produced more flowers (*cf.* Ehrlén & Münzbergová, 2009). After accounting for flower number, we detected insignificant selection on flowering time in 2007, although later plants tended to make more seeds. We observed direct selection for earlier flowering in 2008, the late-snowmelt year—but only at one site (South Gothic). Thus, the differences in environmental conditions between years did not have a strong or consistent effect on the relative success of early- vs. late-flowering individuals, and we cannot conclude that climate warming will lead to strong selection on flowering time in this species. Although warmer springs may be accompanied by a greater risk of frost damage and a consequent overall reduction in seed set, we have no evidence that escape via later flowering will be favoured.

Furthermore, despite the year-to-year variation in abiotic conditions, in neither year did pollinator availability appear to be the major factor limiting seed production, at least not among the early-flowering plants used in our pollen supplementation experiments. However, an absence of pollen limitation at the whole-plant level can mask variation at the level of individual flowers—variation that can shed light on patterns of pollen supply and resource allocation within plants (Wesselingh, 2007). In our study, early flowers tended to be more pollen limited in 2007, and late flowers (at both sites) more pollen limited in 2008. Why did these patterns not translate into pollen limitation at the whole-plant level? The large number of late flowers held in reserve, coupled with flexibility in allocating resources to a subset of successfully pollinated flowers (see below) buffer this species from short-term variation in pollinator availability. Results from this species, therefore, do not provide much support for the hypothesis that the earlier springs expected with future climate change will disrupt plant–pollinator interactions (Price & Waser,

1998; Dunne *et al.*, 2003; Hegland *et al.*, 2009). We did not quantify pollinator visitation at our study sites, but we did note that bumble bees were visiting flowers by the end of the first week of flowering, even in 2007. This may explain the observation that any pollen limitation in early plants was short-lived. Flowers on plants from which pollinators were excluded often lasted five days or more if unpollinated, something that could have played a role in preventing pollen limitation (assuming that stigmas remained receptive). However, we rarely observed such long-lived flowers on open-pollinated plants, even early in the season, suggesting that most flowers were receiving some insect visits.

Why were late-flowering individuals selected against at the South Gothic site in 2008? Declining fecundity in later plants mirrored the pattern of reduced seed set of late flowers within plants, and both may owe something to reduced pollinator visitation later in the season. However, declining resource availability (*e.g.*, soil moisture) might also be responsible. At the Avery site, where there was more topographic variation and a greater number of plants growing in relatively moist microhabitats, we did not observe any decline in seed set in later plants (after accounting for flower number). Direct pollinator observations, or pollen-supplementation experiments with previously unvisited plants exposed to pollinators at different times during the flowering season, would help in understanding temporal patterns of pollinator visitation and pollen limitation in these populations.

Within plants, the decline in seed set and seed mass seen in later flowers in 2008 was probably due mainly to resource limitation. No reductions in seed quantity or quality were observed in 2007, when early flowers set few seed, or in bagged plants on which few flowers were pollinated. Declining fecundity in late flowers has been observed in many plants (Nicholls, 1987; Medrano *et al.*, 2000; Humphries & Addicott, 2004; Kliber & Eckert, 2004), although in some cases it may be due to structural constraints (*e.g.*, distal positioning of late flowers when flowering is acropetal [Diggle, 1997], or declining ovule number in later flowers [Thomson, 1989]). In *Cryptantha flava* (also Boraginaceae), for example, seed set is higher in early flowers, but does not increase in later flowers if early flowers are prevented from setting seed (Casper, 1984). The greater success of early flowers in that species may be due to their location closer to the main axis of the plant, suggesting that resource supply to late flowers, rather than the amount of resources available to the whole plant, is the important limiting factor. In *M. fusiformis*, limited resource availability, and not structural constraints, seems to prevent high seed set and

mass in late flowers when pollen is unlimited. Thus, later flowers probably serve as a back-up in the event of poor pollination of earlier flowers.

Reproductive strategies

Many temperate-region perennials appear to use stored resources to produce as many flowers and fruit as possible early in the growing season. Plant populations often show positively skewed flowering distributions, perhaps because most individuals begin flowering in response to a discrete environmental cue, while the end of flowering is not similarly truncated (Rathcke & Lacey, 1985). In many perennials, larger plants flower earlier (Widén, 1991; Pettersson, 1994; Bishop & Schemske, 1998; Ollerton & Lack, 1998; Lacey *et al.*, 2003; Sandring *et al.*, 2007; Sola & Ehrlén, 2007)—a pattern we also observed here—suggesting that early reproduction is favoured by those plants with sufficient below-ground reserves to allow rapid growth as soon as conditions permit. Flowering distributions at the level of individual plants have received less attention than population-level patterns, but these can be positively skewed, too (Thomson, 1985; Blionis *et al.*, 2001). Finally, allocation of resources within plants also favours early flowers, these almost invariably having higher levels of fruit set if pollination is adequate (Stephenson, 1981; Thomson, 1989; Diggle, 1997; Ladio & Aizen, 1999; Medrano *et al.*, 2000; Humphries & Addicott, 2004; Kliber & Eckert, 2004; Brown & McNeil, 2006; Buide, 2008).

This appears to be the strategy employed by *M. fusiformis* in our study populations, with most plants opening most of their flowers early in the growing season—a pattern that, if anything, was stronger in 2007, the early-snowmelt year. In principle, such a strategy carries with it the risk that poor conditions in early spring (*e.g.*, early-season frosts or pollinator inactivity) might have a devastating impact on the population. However, we did not detect selection on skewness of the flowering schedule in either year, indicating that, at least within the context of currently existing variation in flowering schedules, plants with a strong pattern of preferential allocation to early flowering are not at a disadvantage. Furthermore, although reproductive success was lower in 2007 than in 2008, we found only weak evidence for pollen limitation—and this only in the earliest flowers, suggesting that mismatches with the timing of pollinator activity are not a major threat for this species. Our data also suggest that the possession of a large number of flowers, and the ability to adjust resource allocation among flowers in response to pollination, make *M. fusiformis* somewhat insensitive to variation in environmental conditions. Given the great

interannual variation in snowpack in these habitats, it makes sense that high-elevation plants would have evolved resilience.

Responses to future climate change

There may nevertheless be a limit to how well these strategies buffer the plant against effects of future climate warming. Further reductions in snow accumulation are predicted for western North America (Mote *et al.*, 2005; Christensen *et al.*, 2007), and these may bring an increased risk of frost damage in spring (Inouye *et al.*, 2002; Inouye, 2008) along with decreased soil moisture later in the summer. Because summer drought already appears to be a factor limiting the duration of the growing season for *M. fusiformis*, it is uncertain to what extent plants will be able to maintain seed set by reallocating resources to later flowers if early flowers fail. Plasticity in the duration of flowering and the pattern of resource allocation among flowers may prevent large fluctuations in reproductive success despite climatic variation; this (along with a long generation time) may limit the capacity for evolutionary change in flowering phenology, even if the trait is strongly heritable. Indeed, we did not detect consistent selection on flowering schedules in either year of our study, despite the dramatic differences in climate between the two years. On the other hand, future, more extreme variation in climate has the potential to produce novel phenotypes through plastic changes in flowering patterns; these would then be exposed to selection and might provide material for future adaptation (Price *et al.*, 2003; Donohue, 2005). The interaction between plastic and evolved responses to climate change deserves further study.

Acknowledgements

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Chapter 4

Seasonal change in a pollinator community and the maintenance of style-length variation in *Mertensia fusiformis* (Boraginaceae)

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“Seasonal change in a pollinator community and the maintenance of style-length variation in Mertensia fusiformis (Boraginaceae)”.

Abstract

In subalpine habitats, patchiness in snow accumulation and timing of spring snowmelt produces marked variation in flowering phenology, even over small spatial scales. Plants in early- and late-melting patches are therefore likely to experience very different conditions during their flowering periods. If this patchiness is consistent across years, and if there is limited gene flow between early and late patches, adaptation to the local flowering time might occur. Here, we tested this hypothesis of “adaptation by time” in *Mertensia fusiformis*, a species that shows pronounced, continuous variation in style length within and among populations. Specifically, we hypothesized that lower air temperatures and higher frost risk would favour short-styled plants (in which stigmas are potentially better shielded from the cold) in early-flowering patches. Conversely, we expected that pollinator communities in late-flowering patches would more effectively pollinate, and therefore favour, long-styled plants. As expected, late-flowering populations experienced warmer temperatures than early-flowering populations and a different suite of pollinators: Nectar-foraging bumble bee queens and male solitary bees predominated early in the season, but the number of pollen-collecting female solitary bees increased dramatically later in the season. Furthermore, we found significant differences among pollinators in their abilities to transfer pollen to stigmas at different heights (*i.e.*, a style-length $\times$ pollinator interaction). Because pollen-collecting bees were relatively ineffective at transferring pollen to short-styled plants, they should select for longer styles if plant fitness is limited by pollen receipt. However, although plants in late-flowering patches tended to have longer styles than those in early patches, this difference was not consistent. Also, nectar-collecting bees were approximately

equally effective on long- and short-styled plants, so their prevalence early in the season would not be expected to favour short styles; nor did we find any difference in temperature- or frost-sensitivity between long- and short-styled plants. Our results suggest that seasonal change in pollinator-mediated selection on style length may help maintain variation in style length, but the prevalence of short styles in these populations requires further explanation.

Introduction

Interspecific interactions vary over a species' geographic range, potentially resulting in a “geographic mosaic” of coevolution (Thompson, 2005)—or, at least, in a mosaic of selective forces acting on one of the interaction partners. The importance of spatial variation in interactions, and resulting selection, is widely recognized (*e.g.*, Herrera *et al.*, 2006; Andrew *et al.*, 2007; Siepielski & Benkman, 2007; Gómez *et al.*, 2008). If gene flow between localities is limited, local adaptation is possible. Local adaptation combined with limited migration is one possible mechanism for the maintenance of genetic variation within individual populations (Mitchell-Olds *et al.*, 2007).

There is a temporal analogy to spatial variation in selection. If a species exhibits variation in reproductive phenology, even within a small area, early-reproducing individuals may never encounter late individuals. Limited gene flow between early and late sub-populations can result in “isolation by time”, analogous to the more widely appreciated isolation-by-distance concept of population genetics (Hendry & Day, 2005). Such isolation may be accompanied by divergent selection pressures acting on early and late sub-populations (Fox, 2003; Hendry & Day, 2005) and, in theory at least, can lead to allochronic speciation (Devaux & Lande, 2008).

Alpine plants often exhibit marked variation in flowering phenology even over small geographic areas due to patchiness in snow accumulation and melt date (Galen & Stanton, 1995; Yamagishi *et al.*, 2005). The locations of late and early patches can be remarkably stable over multiple years (Thomson, 2010), such that individual plants of polycarpic species are fairly consistent in their rank order of flowering across years. If seed dispersal is limited, and pollen transfer occurs only between individuals flowering at the same time, this consistency in relative flowering time can extend across generations—raising the possibility of “adaptation by time” (Hendry & Day, 2005). In particular, flowering earlier or later in the season could mean exposure to different weather conditions as well as a different biotic environment: Patchiness in snowmelt does not

greatly affect the phenology of mobile animals in the community, which is likely to be more affected by other factors (*i.e.*, temperature). Early and late plants are therefore likely to come in contact with different herbivores and pollinators. This small-scale heterogeneity in selection could play a role in the maintenance of phenotypic variation in plant populations.

Mertensia fusiformis (Boraginaceae) is a herbaceous perennial that flowers shortly after snowmelt in subalpine meadows of western North America. It is self-incompatible, insect-pollinated, and lacking mechanisms for long-distance seed dispersal (although its seeds possess an elaiosome attractive to ants; Turnbull *et al.*, 1983). Timing of flowering is strongly influenced by local snowmelt date, such that plants in sheltered, low-lying, or north-facing patches may flower up to a month later than plants growing in more exposed, south-facing patches nearby. Intriguingly, the flowers of this species show pronounced variation in style length (Plate 4.1): Some individuals have stigmas projecting well beyond the anthers, a phenomenon known as approach herkogamy (Webb & Lloyd, 1986), while others have stigmas concealed beneath the ring of anthers (reverse herkogamy).

In addition to the variation among individuals, there also appears to be variation among *M. fusiformis* populations in the distribution of style lengths. This could be due to differences in selection between populations. Specifically, we hypothesized that, over the course of the season, changes in weather (including, for instance, the frequency of damaging frosts) or in the pollinator fauna could impose temporally varying selection on style length. This could generate consistent differences in floral morphology between relatively early- and late-flowering populations—*i.e.*, adaptation by time. This hypothesis depends on style length being heritable in *M. fusiformis*, something for which we currently lack evidence. However, the existence of fixed (and taxonomically important) differences in style length among other *Mertensia* species suggests that the trait has some genetic basis in this genus. For example, the early-flowering, high-elevation *M. brevistyla* has short styles (*i.e.*, reverse herkogamy), while the later-flowering *M. ciliata* has long styles (*i.e.*, approach herkogamy).

We investigated the following two complementary hypotheses to explain existing patterns of style-length variation in *M. fusiformis*:

Hypothesis 1: Variation in temperature and risk of frost favours different style lengths at different times of the season. There are two elements of this hypothesis. (A) Early-flowering

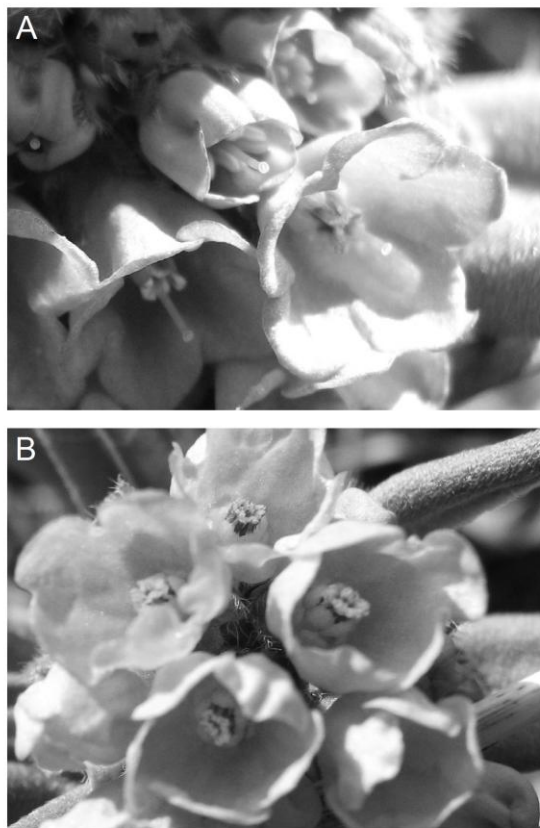


Plate 4.1. Inflorescences of *Mertensia fusiformis*, showing variation in style length. (a) Approach herkogamy; (b) reverse herkogamy.

populations may be more likely than late ones to experience frost, which can damage open flowers, preventing seed set (*i.e.*, destroying female function). Frost due to radiative heat loss may pose a greater threat to long styles than to short styles, the stigmas of which are shielded by the anthers and corolla-tubes. Therefore, if frost damage is indeed more likely early in the season, we expect short-styled plants to be favoured in early-flowering populations. (B) Even in the absence of tissue damage by frost, cold temperatures could affect chances of ovule fertilization by slowing or preventing pollen-tube growth in styles (Lankinen, 2001; Hedhly *et al.*, 2005; Seymour *et al.*, 2009); this too would tend to favour short styles in early-flowering populations.

Hypothesis 2: Variation in the suite of available pollinators favours plants with different style lengths at different times of the season. In our study area, *Bombus* queens are the most conspicuous potential pollinators for early-spring flowers. *Bombus* queens visiting *M. fusiformis* appear to focus mainly on nectar collection. Males of certain solitary bee species, most of which are protandrous, also appear in early spring; those with long tongues (*e.g.*, *Osmia* spp.) can reach the nectar in *M. fusiformis* flowers. Female solitary bees emerge later in the season, and may visit *M. fusiformis* for nectar (if long-tongued), for pollen (regardless of tongue length), or both. Hence, relatively late-flowering *M. fusiformis* may receive more visits from smaller, pollen-collecting bees. It is reasonable to expect different selective pressures to be exerted by the different types of pollinators, which differ markedly in size and foraging behaviour. In particular, compared to nectar-collecting bees, which must force their mouthparts to the base of the flower, pollen-collecting solitary bees would be less likely to contact the stigmas of short-styled plants. If this is the case, and if female solitary bees are in fact more important pollinators late in the season, we expect long-styled plants to have more pollen deposited on stigmas in later-flowering populations. If greater pollen deposition results in increased fitness through seed set, long styles should be favoured in late populations.

Here, we present tests of these two hypotheses using field experiments and observations. We collected data from multiple populations on style length, ambient temperatures during flowering, and flower visitors, to determine whether differences consistent with our hypotheses exist between early- and late-flowering sites. We then experimentally tested whether particular weather conditions or pollinators could differentially affect the female reproductive success of

plants with different style lengths. Our results provide a partial explanation for the existing style-length variation in *M. fusiformis*.

Methods

Study organism

Mertensia fusiformis Greene (“alpine”, “dwarf”, or “spindle-root bluebells”) grows abundantly in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL), near Crested Butte, Colorado, USA, where it typically begins flowering 7–14 days after snowmelt (Inouye *et al.*, 2000). Individual flowering plants are ~10 cm tall and commonly produce approximately 50 flowers (although more than 900 flowers, on multiple stems, are possible). Flowers are ~1 cm long, blue, pendent, and campanulate. Stamens have short, flattened filaments arising from the junction of the corolla-tube and limb, and the filaments and anthers together form a cone around the style, making the nectaries at the base of the corolla-tube inaccessible to short-tongued insects. The plants are self-incompatible (Chapter 3) but compatible with other plants of similar style length (*i.e.*, there is no heteromorphic incompatibility; J. Ogilvie, unpublished data).

Study sites

We selected 14 sites in the vicinity of the RMBL, comprising seven pairs of “early” and “late” sites (Appendix B). Paired sites were closer to each other than to other sites, but were separated from each other by up to 2.8 km and 187 m in elevation (on average, 1.0 km and 40 m). At each site, we set up an observation plot of approximately 300 m² within which we monitored flowering phenology and observed pollinators (see below). To obtain an unbiased sample of individual plants, we established 4–6 belt transects bisecting each plot, and flagged *M. fusiformis* plants with ≥ 5 buds growing inside the transects; transect widths were adjusted according to plant density so that the number of plants would total approximately 30. These 30 plants were used to define flowering phenology and style-length distribution within the plot. Peak *Mertensia fusiformis* flowering, defined as the first date on which the maximum number of flagged plants were in flower, occurred from 11 to 27 (mean = 16) days later at late sites compared to their early counterparts. We placed a Hobo (Onset Computer Corp., Bourne, MA, USA) or LogTag (MicroDAQ.com, Contoocook, NH, USA) data-logger in a shaded location within 100 m of each

plot to record air temperatures during the flowering period. At a subset of these sites, plants growing up to 50 m outside the observation plots were used for the experiments described below.

Floral morphology

To verify that there is less variation in style length within than among individuals, and that style length is not simply a function of flower rank or position, we selected 30 plants for repeated flower-sampling. Near each of three “early” study plots, we haphazardly chose (without regard to style length) 10 plants that had at least 20 flower buds each. We collected two open flowers per plant (if possible) on each of four separate occasions throughout the flowering period of each plant, spanning a period of approximately two weeks. If plants had multiple stems, the two flowers were chosen from different stems. To characterize floral morphology at each study site, we also collected one haphazardly selected open flower from each of the 30 flagged plants in each observation plot. For each flower, we measured corolla length, stigma height (from the base of the superior ovary to the top of the stigma), and anther height (from the base of the ovary to the top of the highest anther); from these measurements we calculated herkogamy as *stigma height* – *anther height*. We also measured total stem length (the summed lengths of all the plant’s stems) as an index of plant size.

Although *M. fusiformis* shows continuous variation in stigma height and stigma–anther separation rather than discrete style-length morphs, for all experiments described below we categorized plants as either “long-styled” (*i.e.*, stigma exerted beyond tips of anthers; herkogamy) or “short-styled” (stigmas below bottom of anthers), based on inspection of a small number of open flowers. Plants falling between these two categories (*i.e.*, with stigmas overlapping anthers) were omitted from experiments because we were primarily interested in detecting functional differences between the extreme phenotypes, rather than in characterizing the shape of selection on style length.

Floral temperature measurements

To determine whether stigmas of long- and short-styled plants experience different temperature regimes, we used thermocouples (type T, 36-gauge copper–constantan) attached to flowers during several clear nights. To minimize microsite variation in temperature associated with different plants, we selected only long-styled plants for these measurements, using a paired

design in which one flower per plant was assigned to be “long” and a matched flower on the same plant was assigned to be “short”. (Taking both “long” and “short” measurements on the same flower would have been preferable but was logistically impossible.) We looped a thermocouple around the end of the style of the “long” flower to obtain temperatures close to the stigma, and we inserted a second thermocouple into the “short” flower to measure temperatures within the corolla tube, where the stigma of a short-styled plant would be situated. Data-loggers (OM-CP-QuadTemp, Omega Engineering Inc., Stamford, CT, USA) recorded temperatures every 10 minutes between 1900 h and 0900 h the following morning. We used a total of 15 plants, and recorded temperatures over five nights (2–4 plants per night).

Post-pollination temperature experiment

We used natural temperature variation to test whether low temperatures, by slowing the rate of pollen tube growth down styles, would negatively affect seed set in long-styled plants. For this experiment, we first removed all wilted and open flowers from 20 long and 20 short plants to prevent resource pre-emption by early fruits. We then covered plants with pollinator-exclusion bags (1 mm mesh). As new flowers opened over the following nine days, matched pairs of flowers on each plant were assigned to morning- and evening-pollination treatments and marked accordingly. “Morning” flowers were cross-pollinated by hand between 1000 h and 1130 h, when we expected air temperatures to continue to rise. We used two different plants as pollen donors for each recipient flower. “Evening” flowers were pollinated in the same way between 1825 h and 1930 h, to correspond with sunset at our site. All hand-pollinations were conducted during conditions in which bumble bees normally forage. In total, a minimum of four “morning” and four “evening” flowers were pollinated on each plant (one of each treatment per day on at least four days) between 23 and 31 May. Plants remained bagged until seed set of all marked flowers had been recorded.

Frost experiment

We used a natural frost event on the night of 24 May 2010, when air temperature dropped to approximately -5°C , to test whether freezing temperatures differentially impair the functioning of long and short styles. On the day before the frost, we selected 20 short and 20 long plants and clipped all wilted flowers. We then marked up to five open flowers per plant as a “frost” treatment. On the morning after the frost, we cross-pollinated as many of the marked flowers as

possible to ensure that pollen supply was not limiting fruit set. (Flowers that were visibly damaged by frost were not pollinated and were omitted from analysis.) We then marked up to five newly opened flowers (that had not been open during the frost) or unopened buds on the same plants to serve as paired controls; these were cross-pollinated in the same way over the following days as they opened. Minimum temperatures on subsequent nights stayed above -2°C . Seed set of all marked flowers was recorded.

Both the temperature and frost experiments were set up in a paired design, with both treatments applied to each individual plant. However, extensive herbivore damage to developing fruits meant that many plants were missing one treatment or the other. We therefore analysed both experiments as simple two-factor ANOVAs to maximize our sample size. Evaluating the effect of style length on within-plant differences between treatments with *t*-tests (*i.e.*, taking advantage of the paired design, but with reduced sample sizes) gave qualitatively identical results.

Pollinator surveys

We surveyed the pollinator community at each site by spending a minimum of five person-hours (up to eight, if pollinators were scarce), observing insect visits to *M. fusiformis* within the observation plots. Surveys took place in good weather on at least five separate days, during a time interval spanning four days before peak flowering to nine days after peak flowering in the patch. During this time, a minimum of 60% (mean = 96%) of all flagged plants in the plot were simultaneously in flower. All insects observed entering flowers to obtain either pollen or nectar were considered potential pollinators. We attempted to follow each insect for its entire foraging bout. To characterize the pollinator fauna, we counted the number of foraging bouts by each taxon of flower visitor. Most insects were identified to family or genus in the field, and sex or caste of all bees was recorded. Most *Bombus* individuals could be identified to species. A small number of flower visitors were collected for more precise identification.

We categorized visitors as pollen-collectors or nectar-collectors. All *Bombus* queens visiting *M. fusiformis* appeared to be collecting nectar exclusively (most did not have pollen loads and showed no sign of active pollen collection). Male bees, all butterflies, and all long-tongued bombyliid flies were assumed to be visiting for nectar. In contrast, short-tongued bees (Halictidae and Colletidae) and syrphid flies were unable to reach the nectaries, but were clearly collecting or consuming pollen. Female *Osmia* can reach *M. fusiformis* nectaries and often enter

flowers for nectar. However, because the majority of female *Osmia* were carrying pollen loads, or were actively collecting pollen, we categorized all visits by female *Osmia* as pollen visits.

Pollinator effectiveness

To test the effectiveness of different types of pollinators at transferring pollen to stigmas of short- and long-styled plants, we allowed freely foraging insects to make single visits to unvisited plants. These plants had previously been covered with pollinator-exclusion bags and categorized as short or long (again excluding plants of intermediate style length). We removed bags and observed plants until they were visited. The visitor's identity and the number of visited flowers were noted, and the visited flowers on each inflorescence were marked. In some cases, we could not tell which individual flowers within a dense inflorescence had been visited, so all open flowers were marked. Seed set was calculated as *total number of seeds produced by marked flowers* $\div$ (*number of visited flowers* $\times$ *ovule number per visited flower*), where per-flower ovule number was typically four. We obtained mean seed mass (an average across all mature seeds resulting from a single pollinator visit) as an additional measure of pollinator quality. The individual plant was considered the unit of replication.

To increase our sample size of plants visited by *Bombus* queens (which were scarce at this site at the time of our experiments), we used five *B. bifarius* queens that had been kept in captivity for four weeks on a diet of artificial nectar and prepared pollen for use in an unrelated experiment. We chilled these bees and then released them individually into a portable flight cage (approximately 1 m diameter $\times$ 1 m height) placed over a small number of previously bagged plants. We allowed each bee to probe at least one virgin flower before visiting the experimental plants to ensure the possibility of cross-pollination. Each bee visited between two and six plants, each of which was treated as an independent replicate.

Visitors were divided into four categories for analysis: 1) *Bombus* queens (specifically, *B. bifarius*, *B. melanopygus*, and *B. sylvicola*; all are “short-tongued” bumble bees); 2) male *Osmia*; 3) female *Osmia*; and 4) female Halictidae (*Halictus virgatellus* and *Lasioglossum* [*Evylaeus*] sp.). Visits by the first two were considered nectar visits, and those by the latter two pollen visits (as above). Rarer visitors (*Colletes*, syrphids) were not included because of low sample sizes (≤ 3). This gave us a fully crossed (4 pollinator types, or 2 visit types, $\times$ 2 style lengths) design.

Analysis

All analyses were conducted in R v. 2.7.2 (R Development Core Team 2008). We used Type III sums of squares (in the `car` package) for all ANOVAs. Response variables that were proportions were arcsine transformed, and other variables were 4th-root- or log-transformed, to achieve normality whenever possible. We used non-parametric comparisons in cases where a large number of zero values made transformation ineffective.

Among-site differences in herkogamy were tested using a general linear mixed model (package `lme4`), with “early/late” as a fixed predictor and “site” as a random predictor. Significance of the early/late factor was evaluated using a likelihood-ratio test of nested models.

Our pollinator effectiveness experiment was set up as a two-factor ANOVA, but the large number of zero values for seed set meant that parametric analysis was potentially inappropriate. (Seed masses, however, were normally distributed.) We nevertheless ran the ANOVA (on arcsine-transformed data) to test for the interaction effect. We then followed up with non-parametric Kruskal–Wallis tests for the specific one-way comparisons of interest.

Results

Floral morphology

Across a sample of 30 plants, mean corolla length, stigma height, and anther height were all positively correlated (Pearson’s $r > 0.65$, $P < 0.0001$). Mean stigma height was more variable than the other traits (among-plant coefficient of variation = 14.5%, vs. 8.5 and 9.8% for corollas and anthers). Herkogamy was correlated with stigma height ($r = 0.68$, $P < 0.0001$) but not with anther height ($r = 0.02$, $P = 0.91$), indicating that among-plant variation in herkogamy was primarily due to variation in stigma height. None of the floral measurements was correlated with plant size (measured as $\log_e$ [total stem length]; all $|r| < 0.12$, $P > 0.55$). There were significant differences among individual plants in the degree of herkogamy ($F_{29,204} = 24.4$, $P < 0.0001$), but the sample as a whole showed a fairly continuous, close-to-normal distribution (Fig. 4.1).

Floral morphology differed significantly among study plots ($F_{13,401} = 5.3$, $P < 0.0001$; Fig. 4.2). Plants in late-flowering plots tended to have more exerted stigmas, but this trend was not

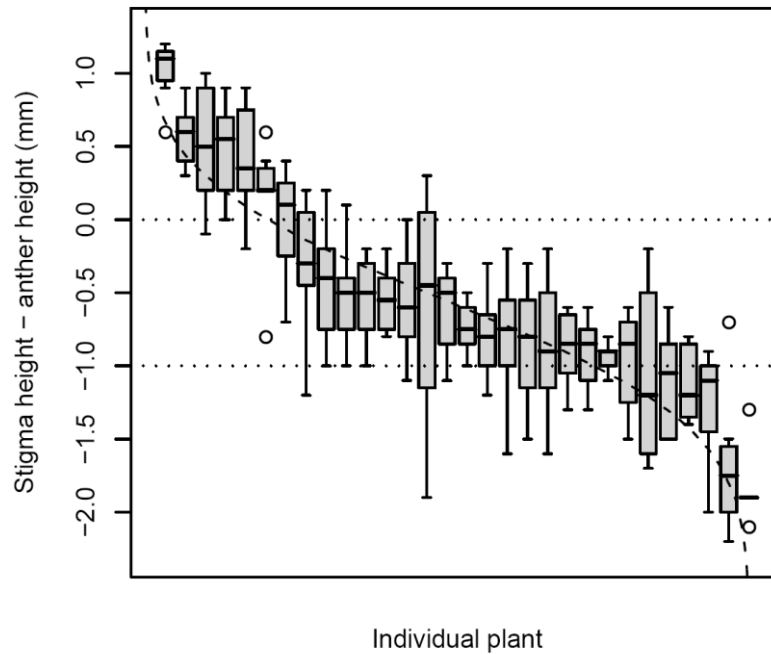


Figure 4.1. Tukey boxplots showing variation in herkogamy within and among 30 individual *Mertensia fusiformis* plants. Plants are plotted in order of decreasing mean stigma exsertion. Boxes show within-plant medians and interquartile ranges. The dashed line was generated by drawing many random values from a normal distribution with mean = -0.56 and s.d. = 0.67 and plotting the sampled values in descending order. Plotted values were connected with a spline. Values between 0 and -1 , demarcated by the two horizontal dotted lines, indicate overlap between stigmas and anthers (*i.e.*, no herkogamy), assuming anthers are 1 mm long (an approximate value; not measured in all flowers). Differences in herkogamy among individual plants are significant. $N = 5-8$ flowers per plant.

significant (likelihood-ratio $\chi^2 = 5.7$, $df = 3$, $P = 0.13$; Fig. 4.2), and the trend was reversed at two site pairs.

Environmental conditions at early and late sites

During the period spanning our pollinator observations at each site, early sites were significantly cooler, on average, than late sites (mean temperature 9.7 vs. 12.1°C; paired t -test, $t_6 = 6.9$, $P = 0.00046$). Early sites were also more likely to experience a severe frost: Three of seven early sites had minimum temperatures $< -4^\circ\text{C}$, whereas no late sites experienced temperatures below -1°C .

We observed 117 visitors to *M. fusiformis* flowers (on average, 2.4 per observer-hour) at early sites and 452 visitors (10.0 per observer-hour) at late sites (Table 4.1, Appendix C). This almost certainly represents a higher per-flower visitation rate at late sites, but we cannot be sure without estimates of floral density at each site. Early sites had a higher proportion of visitors foraging for nectar than late sites (70% vs. 39%; paired t -test, $t_6 = 2.7$, $P = 0.034$). Late sites were instead dominated by pollen-collecting bees (Fig. 4.3). This change was mainly driven by the greater number of visits by pollen-collecting female *Osmia* and halictids in late sites (mean 0.62 *Osmia* and 0.36 halictid visits per site per observer-hour in late sites, vs. 0.035 and 0.077 visits per observer-hour in early sites), rather than to a decline in the number of visits by *Bombus* queens or male *Osmia*.

Temperature and its effects in long and short styles

Contrary to expectations, we found no difference in minimum or mean overnight (2000–0600 h) temperatures between “long” (exposed) and “short” (shielded) stigmas (paired t -tests, $t_{14} < 1.5$, $P > 0.15$); in fact, long stigmas had slightly higher mean and minimum temperatures than shorts (0.7 and -2.7°C for long, vs. 0.6 and -2.9 for shorts). Evening temperatures (1900–2100 h) also did not differ between long and short stigmas (paired $t_{14} < 0.1$, $P > 0.9$).

Daytime (1200–1600 h) air temperatures were consistently higher than night-time (2000–0000 h) temperatures at our study site during the post-pollination temperature experiment (mean difference = 9.8°C). However, we found no effect of the timing of pollination (*i.e.*, morning or evening) on seed set (two-factor ANOVA, $F_{1,76} = 0.21$, $P = 0.65$); nor did we find any interaction between style length and post-pollination temperature ($F_{1,76} = 0.18$, $P = 0.67$).

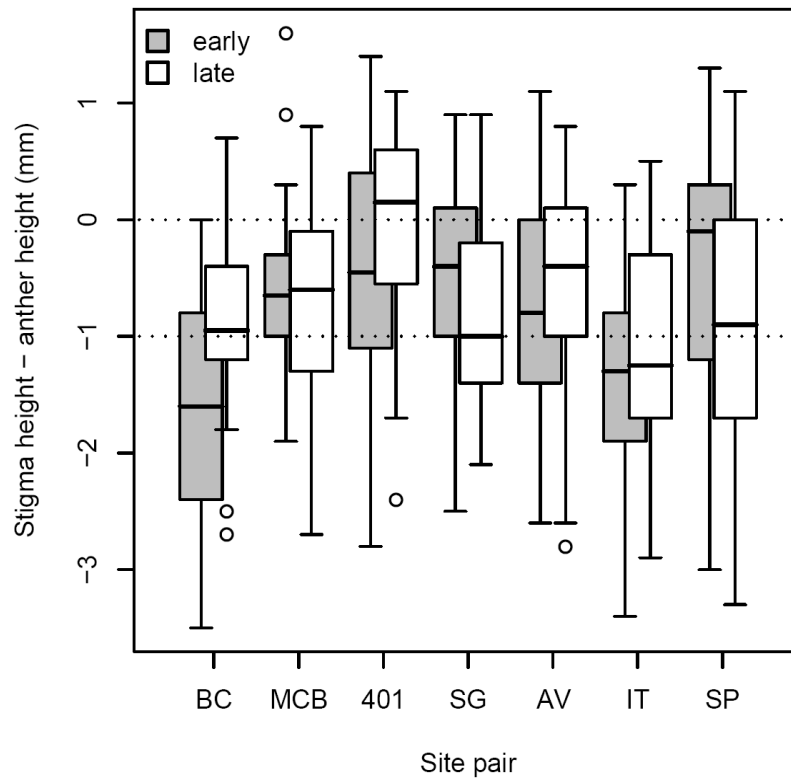


Figure 4.2. Boxplots of herkogamy for plants in the seven pairs of study plots. Early-flowering plots are represented by shaded boxes; late-flowering plots are unshaded. Site pairs are plotted in order of increasing peak flowering date of early plots. Dotted lines as in Fig. 4.1. Early and late plots do not differ significantly. $N = 29\text{--}30$ plants per plot.

Table 4.1. *Mertensia fusiformis* flower visitors recorded in observation plots. See Appendix C for the full species list.

Visitor	Sex/ Caste*	Total in early plots	Total in late plots
Diptera			
Bombyliidae		2	0
Muscoidea		0	2
Syrphidae		3	20
Hymenoptera			
Apidae			
<i>Bombus appositus</i>	Q	1	0
<i>Bombus bifarius</i>	Q	28	10
<i>Bombus bifarius</i>	W	0	1
<i>Bombus californicus</i>	Q	2	1
<i>Bombus centralis</i>	Q	0	1
<i>Bombus flavifrons</i>	Q	6	30
<i>Bombus frigidus</i>	Q	1	0
<i>Bombus mixtus</i>	Q	0	1
<i>Bombus sylvicola</i>	Q	0	3
<i>Bombus</i> sp.	Q	6	2
unknown	M	1	0
Colletidae			
<i>Colletes</i> sp.	F	0	1
Halictidae	F	26	114
Megachilidae			
<i>Osmia</i> spp.	M	28	67
<i>Osmia</i> spp.	F	12	198
Lepidoptera			
Hesperiidae		1	0
Pieridae		0	1

*Note: Sex/Caste recorded only for Hymenoptera. W = worker, Q = queen, M = male, F = female.

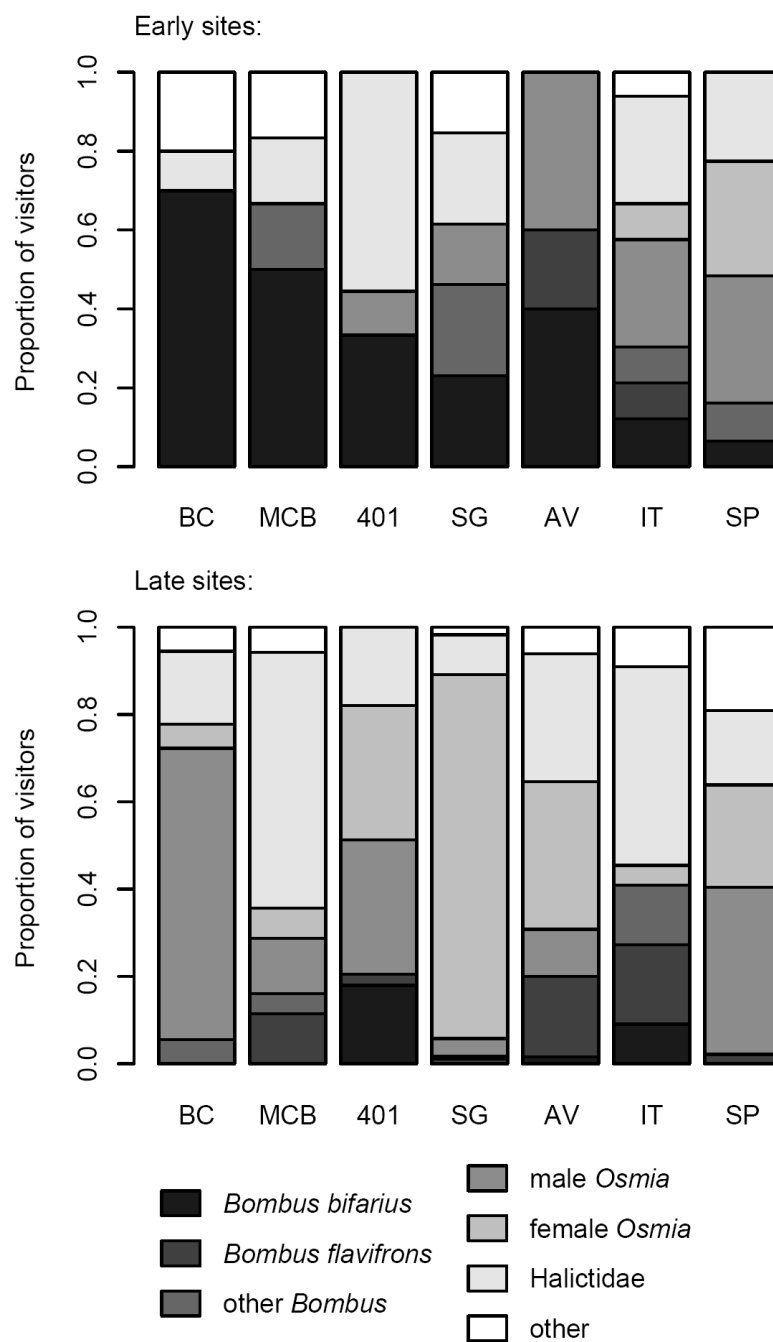


Figure 4.3. Flower-visitor community composition at each of the 14 study plots. Bumble bees (darker bar segments) are responsible for a larger proportion of visits in early sites. Solitary bees (lighter grey bar segments) make up the majority of visits in late sites.

Freezing temperatures before pollination did have a negative effect on ovule fertilization, indicating that our “frost” treatment was effective: seed set of even apparently undamaged flowers was reduced from 55% to 37% on average ($F_{1,72} = 4.64$, $P = 0.035$). This was due to fewer frosted flowers setting fruit compared to unfrosted controls ($F_{1,72} = 3.98$, $P = 0.050$; Fig. 4.4), and not to any reduction in the number of seeds per flower among those that did set fruit ($F_{1,65} = 0.40$, $P = 0.53$; Fig. 4.4). Short-styled plants set significantly fewer seeds than long-styled plants ($F_{1,72} = 4.28$, $P = 0.042$), suggesting that we or the plants’ natural pollinators were less effective at pollinating short styles. Again, however, there was no style $\times$ treatment interaction ($F_{1,72} = 0.06$, $P = 0.80$)—a result that is consistent with the lack of detectable temperature difference between long and short stigmas.

Pollinator effectiveness on long and short styles

Interestingly, seed set from single pollinator visits depended on the interaction between pollinator type and style length ($F_{3,83} = 3.25$, $P = 0.026$; Fig. 4.5). Female *Osmia* and halictids were significantly less effective at transferring compatible pollen to short- than to long-styled plants (Kruskal–Wallis $\chi^2 = 8.25$, $N_{\text{long}} = 19$, $N_{\text{short}} = 16$, $P = 0.0041$ for *Osmia*; $\chi^2 = 4.27$, $N_{\text{long}} = 6$, $N_{\text{short}} = 9$, $P = 0.039$ for halictids). In contrast, *Bombus* queens were equally effective at pollinating both types of plants ($\chi^2 = 0.71$, $N_{\text{long}} = 11$, $N_{\text{short}} = 13$, $P = 0.40$). Male *Osmia* tended to produce higher seed set on short- than long-styled plants (44% on average vs. 29%; Fig. 4.5), but this trend was not significant ($\chi^2 = 1.03$, $N_{\text{long}} = 6$, $N_{\text{short}} = 11$, $P = 0.31$).

Because of low seed set in certain pollinator $\times$ style length combinations (specifically, male *Osmia* on long styles and halictids on short), we have low confidence in some of our seed mass estimates, and low power to detect treatment effects. Trends in mean seed mass were nevertheless similar to those for seed set (Fig. 4.5); however, there was no detectable effect of style length on seed mass for any pollinator or visit type ($P > 0.3$).

Discussion

Patterns of variation in floral morphology

In *Mertensia fusiformis*, variation in herkogamy is a result of continuous variation in style length, without the discrete morphs and inverse variation in anther height that is characteristic of truly heterostylous plants. While the latter type of stylar polymorphism is purely genetically controlled

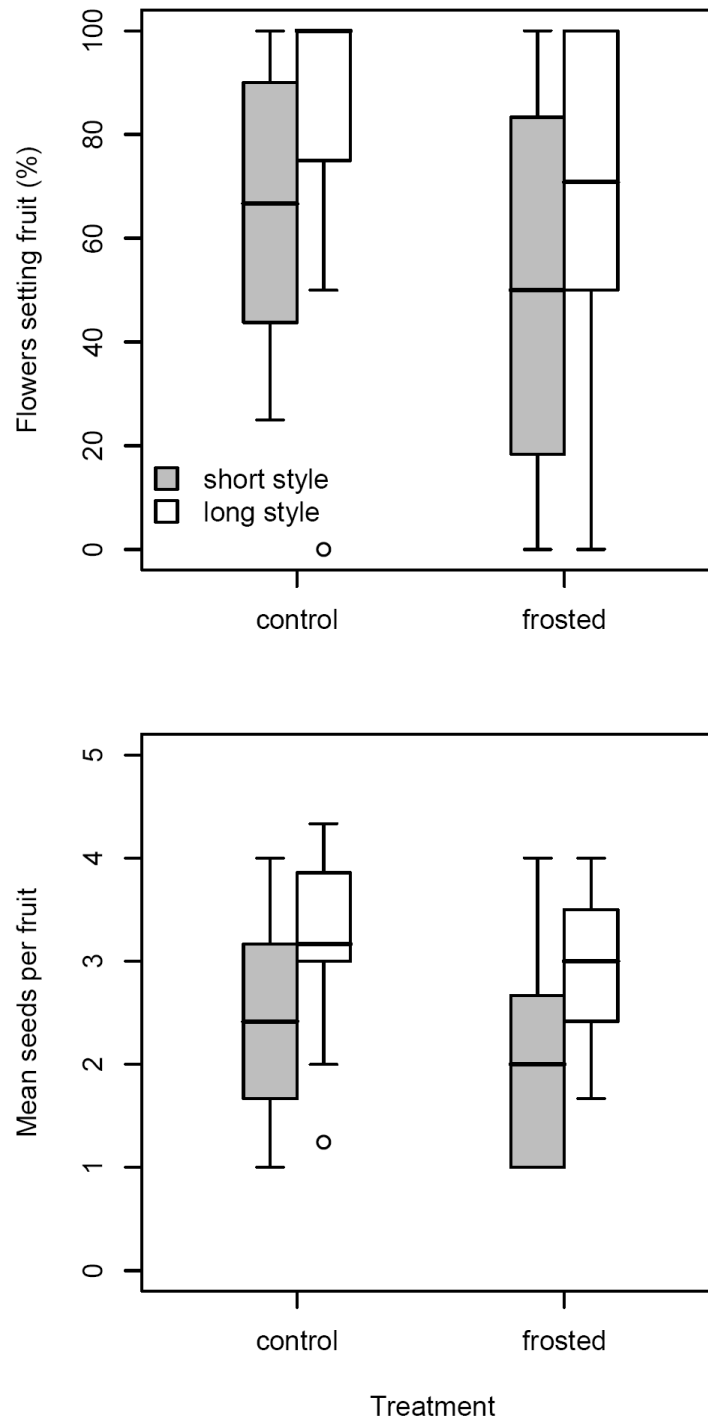


Figure 4.4. Boxplots showing the percent of flowers setting seed, and the mean number of seeds per flower (excluding those that set no seed), for flowers that had either experienced a severe frost the night before (“frosted”) or not (“control”). There are significant main effects of style length and treatment, but there is no significant interaction. $N = 15\text{--}20$ for plants per treatment combination.

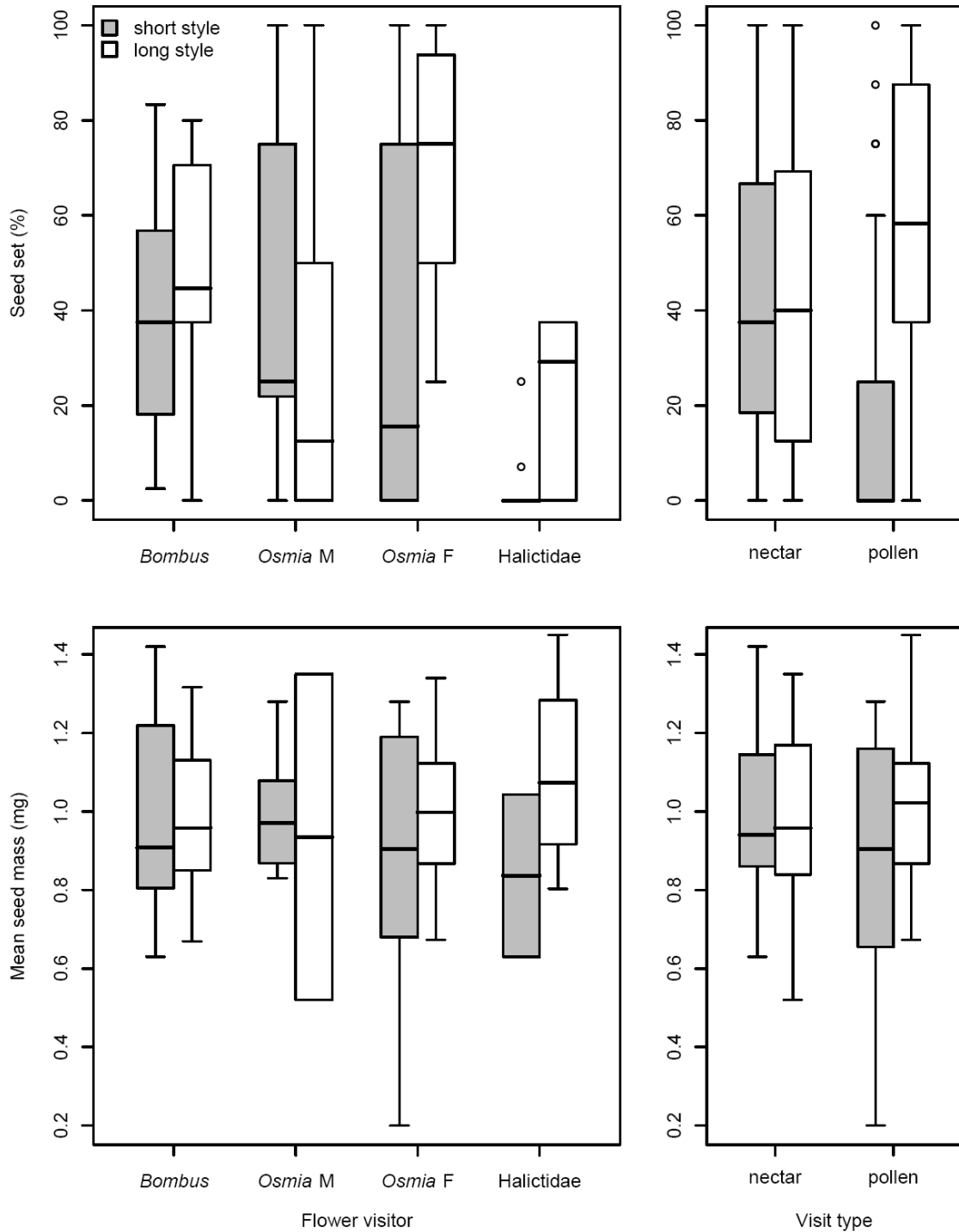


Figure 4.5. Boxplots illustrating effectiveness of different pollinator taxa, and different visit types, at fertilizing ovules of long- and short-styled plants. There is a significant style length $\times$ pollinator type or visit type interaction for seed set, but not for mean seed mass. $N = 6\text{--}25$ plants per treatment combination for seed set; $N = 2\text{--}18$ for seed mass.

(by simple Mendelian inheritance; Lloyd & Webb, 1992), we do not yet know what causes *M. fusiformis* plants to differ in style length or stigma–anther separation. However, continuous variation in style length in other species is often strongly heritable (Kulbaba & Worley, 2008 and references therein). The fact that herkogamy is consistent within individual *M. fusiformis* plants and does not scale with plant size leads us to expect that variation in this species, too, has a genetic basis, but we have yet to confirm this with the necessary quantitative genetic experiments.

Similar variation in style length and herkogamy to that documented here has been reported in *Ipomopsis aggregata* (Waser & Price, 1984); in this species, the variation is correlated with functional gender (longer-styled individuals having shorter corollas and a longer pistillate phase; Campbell, 1989). We did not find any indication that longer-styled plants are more functionally female in *M. fusiformis*; rather, style length, anther height, and corolla length are all positively correlated in this species, and flowers are essentially adichogamous and invariant in ovule number. We therefore suspect that variation in stigma–anther separation is not simply a consequence of variation in a correlated trait.

We have instead hypothesized that this variation is due to temporally or spatially varying selection via female function (*i.e.*, seed set). This may seem incompatible with the finding that *M. fusiformis* in our study area were not strongly pollen limited in 2007–2008 (Chapter 3), since an absence of pollen limitation suggests that selection to maximize pollen receipt should be weak at best. (Note that selection to protect stylar tissue from damage by frost should operate regardless of whether seed set is pollen limited.) However, multi-year studies frequently show that pollinator abundance and pollen limitation vary from year to year (*e.g.*, Herrera, 1988; Baker *et al.*, 2000; Goodwillie, 2001; Buide, 2006); in our study area, occasional years with a dearth of pollinators could drive selection for increased pollen deposition. Furthermore, our earlier study did not investigate whether pollen limitation was influenced by herkogamy and date of first flowering.

Based on our present results, we would predict pollen limitation to be most pronounced in late-flowering plants with short styles: Later flowering periods correspond with the flight season of pollen-collecting female solitary bees, which are comparatively unlikely to contact concealed stigmas. All else being equal, this should select for more exerted stigmas in late-flowering

populations. In contrast, in early populations, visited mainly by bumble bee queens and male solitary bees, most flower visitors will contact both short and long stigmas as they probe for nectar. Hence, pollinator-mediated selection on style length should be weak or absent in early-flowering populations.

However, despite a trend for longer styles in later populations, we were unable to explain most of the variation in style length among populations on the basis of flowering time, and, therefore, as an adaptive response to the seasonal transition in the pollinator community. There are several possible reasons for this. First, differences in selection between early and late populations may be insufficiently strong or consistent to produce measurable differences in stigma–anther separation. Inconsistent selection could result from interannual differences in spatial patterns of snow accumulation, such as those resulting from occasional avalanche runs. Even if spatial patterns of snowpack are consistent, however, among-year differences in the relative timing of plant flowering and insect emergence, or simply in the population sizes of different pollinator taxa, could produce interannual fluctuations in the pollinator community experienced by each *M. fusiformis* population. We are fairly confident that the qualitative pattern we have documented here (of proportionally more nectar visits early in the season) would be consistent, both because of the spatial replication in the present study and because most of the seasonal change in the pollinator community is the inevitable consequence of protandry (early male emergence) in solitary bees. However, quantitative changes in the relative abundances of bumble bees and solitary bees, for example, could change the strength of selection on floral traits. Second, seed- or pollen-mediated gene flow between patches could override differences in selection gradients and prevent morphological differentiation between populations. Seed dispersal from patches with different flowering times might explain the reversal of the expected pattern in style length at our “SP” sites, both of which—unlike most other sites—had moderately bimodal frequency distributions of stigma exertion. Both gene flow and weak selection would tend to prevent or obscure local adaptation.

How are short styles maintained?

Our study addresses not only the issue of adaptation of local populations to seasonally changing conditions, but also a broader question about how genetic diversity is maintained within populations (Mitchell-Olds *et al.*, 2007): Are seemingly maladapted phenotypes present because

they are being constantly generated by mutation? Or do they result from some form of balancing selection that favours different phenotypes in different conditions? If the latter, is it a case of temporally varying selection within individual populations, or of local adaptation and migration of genotypes between populations (*i.e.*, migration–selection balance, or balancing selection at a larger scale; Mitchell-Olds *et al.*, 2007)?

A significant interaction between pollinator type and floral morphology is a necessary condition for pollinator-mediated adaptive diversification in floral form, but fewer studies have succeeded in detecting such an interaction than would be expected from the level of interest in angiosperm diversity (Wilson & Thomson, 1996). Most examples of strong pollinator $\times$ flower type interactions (*e.g.*, Schemske & Bradshaw, 1999; Aigner, 2004; Muchhala, 2007; Streisfeld & Kohn, 2007) have come from contrasts between very dissimilar pollinator types (*e.g.*, bats vs. hummingbirds, birds vs. bees). Our study is unusual in documenting a difference in effectiveness between relatively closely related pollinators visiting naturally co-occurring floral variants (though see also Harder & Barrett, 1993). Clearly, morphological or behavioural differences among bee genera, and between sexes of the same bee species, can have important implications for selection on floral traits. However, it is important to note that a significant pollinator $\times$ flower type interaction is not a *sufficient* condition for diversification, which additionally requires the existence of a trade-off between adaptations to different pollinator types (Aigner, 2001). Our data do not point to a strong trade-off, since we could find no situation in which long styles were inferior. We observed a slight tendency for greater pollination effectiveness of male *Osmia* on short- than on long-styled plants, perhaps indicating that longer styles, which can be pushed outside the ring of anthers during a nectar visit, are less reliably positioned to contact pollen-bearing parts of these insects' bodies. However, seed set from male *Osmia* visits was extremely variable, and the trend was not significant. This leaves us with the challenge of explaining the maintenance of short styles in *M. fusiformis* populations.

It is possible that the observed variation in herkogamy is selectively neutral, as we have not yet measured selection *per se*, but our results suggest that selection for long styles is likely, if only under certain conditions. In fact, we can imagine additional reasons, beyond those investigated here, why stigma exertion might be advantageous. Long styles can potentially facilitate mate choice by females (Mulcahy, 1979; Skogsmyr & Lankinen, 2002). They also seem better suited to avoiding deposition of autogamous pollen (Webb & Lloyd, 1986), which could, in principle,

clog stigmatic surfaces or prevent tube growth from compatible pollen (*e.g.*, Ockendon & Currah, 1977; Parra-Tabla & Bullock, 2005). We previously found that prior or simultaneous deposition of self pollen had no detectable negative effect on seed set in *M. fusiformis*, provided cross pollen is available (A. Gorischek, unpublished data)—suggesting that there may in fact be little or no cost to having a stigma positioned where self-pollen deposition is likely. Nevertheless, receipt of self pollen could have a negative indirect effect by triggering corolla abscission before the flower has been effectively pollinated (Chapter 3), thereby reducing chances of further pollinator visits.

Our hypothesis that short styles might be more protected from radiative heat loss was not supported by either our observational data or the results of the frost experiment. It is possible that small differences in temperature or cooling rate exist between exposed and concealed stigmas, but were not detected using our methods: *Mertensia fusiformis* flowers are too small to permit us to insert thermocouples into the stylar or stigmatic tissue itself, so our measurements may not have precisely captured the relevant temperatures. On the other hand, the typically pendent flower orientation and pubescent leaves of *M. fusiformis* may shield even long styles from heat loss to the night sky (or allow them to benefit from heat radiating from the soil), and our inability to detect cooler temperatures in exerted stigmas may reflect a real lack of thermal cost of longer styles. The fact that a previous night's frost did not reduce seed set of long-styled plants any more than that of short-styled plants supports the latter interpretation. In contrast, it seems inevitable that pollen tubes would require longer to reach and fertilize ovules when starting from a more distant stigma, and that pollen tube growth in both long and short styles would be slower at low temperatures. Nevertheless, we found no evidence (in the post-pollination temperature experiment) that the combination of long styles and cool night-time temperatures reduce seed set. Even during cool nights, pollen of *M. fusiformis* may be quite capable of reaching ovules, regardless of style length. It is worth noting that our experiments were aimed at testing effects of temperature on style tissue and pollen tube growth, but not pollen tube germination, which can also be affected by temperature (Galen & Stanton, 2003); this remains a possible area for future research in our system. Experiments using artificial flowers or the flowers of larger species as models could also help to test how floral orientation and herkogamy affect stigmatic temperature; however, they would be of limited value in determining whether temperature is an important agent of selection in field populations of *M. fusiformis*.

If exposure to a hostile abiotic environment is not a threat to long-styled plants, we must look elsewhere for factors that have maintained short styles in the population. Another possible advantage of shorter styles is that concealment within the corolla-tube may provide protection from herbivory or mechanical damage by pollinators (*cf.* Parra-Tabla & Bullock, 2005). We did not test this possibility here because we have rarely observed herbivore damage to flowers in our study area (notwithstanding the frequent destruction of developing seeds after flowering), and the damage we have observed appears to affect the entire gynoecium and not merely the tip of an exposed style. Nevertheless, the possibility of differential damage to long and short styles does merit further investigation.

In some plants, aspects of stigma morphology may in fact have evolved in response to selection via male function (*i.e.*, pollen export), rather than selection to maximize pollen receipt, a possibility we did not consider here. For example, in *Mimulus aurantiacus*, the large, bilobed stigma closes shortly after receiving pollen—apparently a behaviour that has evolved to minimize interference between male and female function (Fetscher, 2001). One could imagine that short styles might similarly facilitate pollen export in *M. fusiformis*. It is also possible that flowers with protruding styles might deter some prospective flower visitors; if so, the greater likelihood of pollen receipt by long styles (given a pollinator visit) could be offset by a lower probability of receiving a visit in the first place. It seems unlikely to us that the small, unobtrusive stigma of *M. fusiformis* would interfere with pollinator attraction or pollen export, regardless of its placement relative to the anthers. However, tests of pollinator preference, and analyses of pollen removal following single visits to long- and short-styled plants, would be necessary to confirm this.

Conclusions

Early- and late-flowering populations of *M. fusiformis* differ consistently in the set of insects that frequent their flowers. Although our study took place over only a single season, we expect that this qualitative difference would be maintained over multiple years. This seasonal transition in pollinators should favour long-styled plants in later-flowering areas, due to the greater effectiveness of pollen-collecting bees in fertilizing long-styled plants. The observed pattern of among-site variation in stigma exsertion was consistent with this expectation, but the association between flowering time and floral morphology was not significant—perhaps simply due to the

relatively small number of site pairs investigated. Inconsistency among years in population flowering time and immigration from nearby areas with differing floral morphology would be expected to limit our ability to detect local adaptation. The former possibility is easily tested in principle, although it would require several years of observation. The prevalence of non-locally adapted immigrant genotypes is more difficult to assess using direct observations, given the difficulties inherent in tracking seed dispersal. However, these immigrants should be relatively more abundant in smaller populations. Given a sufficient number of study sites, a correlation between adaptation and population size could be tested. Also, because we observed consistent differences in abiotic conditions (temperature) between early- and late-flowering populations, we might predict corresponding adaptations in physiological or morphological traits (other than the floral traits measured here), provided local adaptation is possible. Thus, comparisons of the spatial scale of adaptation in floral and vegetative characters could be revealing. Future investigations along these lines should help us explain the maintenance of within- and among-population variation in floral morphology.

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Chapter 5

An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows

Manuscript to be submitted as Forrest, J. and J.D. Thomson. “An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows”.

Abstract

There are fears that climate change may cause mismatches in the seasonal timing of interacting organisms, owing to species-specific shifts in phenology. Despite concern that plants and pollinators might be at risk of such decoupling, there have been few attempts to test this hypothesis using detailed phenological data on insect emergence and flowering at the same localities. In particular, there are few datasets on pollinator flight seasons that are independent of flowering phenology, because pollinators are typically collected at flowers. To address this problem, we established standardized nesting habitat (trap-nests) for solitary bees and wasps at sites along an elevational gradient in the Rocky Mountains, and monitored emergence during three growing seasons. We also recorded air temperatures and flowering phenology at each site. Using a reciprocal transplant experiment with nesting bees, we confirmed that local environmental conditions are the primary determinants of emergence phenology. We were then able to develop phenology models to describe timing of pollinator emergence or flowering, across all sites and years, as a function of accumulated degree-days. Although phenology of both plants and insects is well described by thermal models, the best models for insects suggest generally higher threshold temperatures for development or diapause termination than those required for plants. In addition, degree-day requirements for most species, both plants and insects, were lower in locations with longer winters, indicating either a chilling or vernalization requirement that is more completely fulfilled at colder sites, or a critical photoperiod before which degree-day accumulation does not contribute to development. Overall, these results suggest that phenologies of plants and trap-nesting bees and wasps are regulated in similar ways

by temperature, but that plants are more likely than insects to advance phenology in response to springtime warming. We discuss the implications of these results for plants and pollinators, and suggest that phenological decoupling alone is unlikely to threaten population persistence for most species in our study area.

Introduction

Numerous authors suggest that climate change may lead to trophic mismatch, or phenological decoupling of interacting organisms (*e.g.*, Price & Waser, 1998; Inouye *et al.*, 2000; Menzel *et al.*, 2006; Cleland *et al.*, 2007; Høye & Forchhammer, 2008). Such effects could arise if, for instance, the spring-time emergence of a herbivorous insect is triggered by different cues than those used by its host plant, and if climate warming differentially affects those cues. In this case, insects might fail to time their emergence to correspond with maximum food availability. There is now abundant evidence of shifts in phenology of individual species in response to climate change (Parmesan, 2007; Rosenzweig *et al.*, 2007), and a small but growing number of documented cases of climate-change-driven asynchrony between adjacent trophic levels (*e.g.*, Edwards & Richardson, 2004; Winder & Schindler, 2004; Both *et al.*, 2006; Visser *et al.*, 2006). However, there is still little beyond anecdotal evidence for temporal mismatches between plants and insect pollinators (Hegland *et al.*, 2009; but see Thomson, 2010).

The lack of evidence may reflect a real absence of asynchrony, since phenologies of both plant and insect species are frequently governed by—or at least well correlated with—air temperatures in the months before emergence or flowering (see below). On the other hand, long-term phenological data suggest that insects (or, specifically, butterflies, the best-studied group) may be more responsive to temperature changes than are many plants (Gordo & Sanz, 2005; Parmesan, 2007). Therefore, if we have not yet observed phenological decoupling of plants and pollinators, this may reflect instead a shortage of the type of detailed, long-term observations upon which these studies are typically based. Such observations are harder to conduct with tiny, mobile, taxonomically challenging invertebrates than with plants and many larger animals. However, it is difficult to draw conclusions about the likelihood of asynchrony on the basis of global generalizations about plants and insects, since species in different environments are likely to have evolved different types of responses to environmental cues. Instead, we will need to understand what factors govern the activity periods of plants and pollinators in particular

localities. In addition, to realistically assess the threat posed by climate change to plant–pollinator relationships, we will need to go beyond simple correlations between temperature and dates of first flowering or first insect sighting, and begin to test the ability of alternative models to predict phenology of interacting species (*e.g.*, Visser & Holleman, 2001). Long-term data and historical records, while extremely valuable as a starting point, are often insufficiently detailed to achieve this more mechanistic goal; so, in many systems, we must use shorter time-series and existing spatial variation in phenology, together with high-resolution climate data, to develop robust phenology models.

Determinants of flowering phenology

Flowering phenology has been relatively well studied, both in genetic model systems (*e.g.*, *Arabidopsis*, *Zea*) and in the field. Many plants respond strongly to photoperiod, with either short or long days promoting bud formation (Glover, 2007). However, temperature also has a strong effect on development rate, and interacts with the photoperiod response to determine the actual timing of flowering (Billings & Mooney, 1968; Ausín *et al.*, 2005; Wilczek *et al.*, 2010). Studies using long-term data on flowering phenology in temperate-zone plants frequently emphasize the temperature effect, showing good correlations between springtime temperatures and dates of first or peak flowering (*e.g.*, Fitter *et al.*, 1995; Miller-Rushing *et al.*, 2007; Miller-Rushing & Primack, 2008). For some species, phenology models have been developed to predict the date of flowering as a function of thermal units (*e.g.*, growing degree-days, the cumulative amount of time spent above a particular threshold temperature), so that warmer years, in which the requisite amount of heat accumulates more quickly, are expected to show earlier flowering (Jackson, 1966; Diekmann, 1996; Wolfe *et al.*, 2005; Hülber *et al.*, 2010).

However, there is another important aspect of plant responses to temperature: Many species have a vernalization requirement, which prevents or delays flowering until the passage of a sufficient period of cold temperatures (Henderson *et al.*, 2003). This chilling requirement complicates interpretation of temperature effects on phenology; in some cases, springtime phenology may even be delayed by warming temperatures because of the reduction in chilling (*cf.* Zhang *et al.*, 2007; Morin *et al.*, 2009).

Finally, in high-altitude and high-latitude environments, flowering time is often strongly correlated with snowmelt (Inouye & Wielgolaski, 2003; Kudo, 2006; Ellebjerg *et al.*, 2008). It is

unclear whether snowmelt truly acts as a cue for later phenological events or whether it simply sets a lower limit on the date of initiation of growth—with the rate of subsequent development then more directly regulated by air and soil temperatures. Testing the separate influences of air temperatures and snowmelt is challenging, both because the two variables are rarely independent, and because, in long-term datasets, only one of the two variables may have been recorded. Field studies that have examined the explanatory power of both snowmelt date and temperature in regulating flowering phenology have not reached consistent conclusions about which factor is of primary importance (Thórhallsdóttir, 1998; Dunne *et al.*, 2003; Hülber *et al.*, 2010). This is perhaps unsurprising, given the differences among studies in the species considered, the magnitude of variation in melt dates, and the statistical methodology. However, it demonstrates that in spite of the wealth of knowledge about flowering induction in model species such as *Arabidopsis thaliana*, we are still a long way from understanding what regulates flowering time of other taxa in the field.

Determinants of insect phenology

In many insects, development rate is closely tied to temperature (Tauber *et al.*, 1986; Gullan & Cranston, 2000). More specifically, the expected stage of development at a given time (*e.g.*, eclosion, pupation, or adult emergence) can often be predicted from the accumulated degree-days above some threshold temperature up to that point (reviewed by van Asch & Visser, 2007). The threshold (base) temperature may be explicitly tested (*e.g.*, Bryant *et al.*, 1997; Manel & Debouzie, 1997; Régnière *et al.*, 2007; White *et al.*, 2009) or simply assumed (*e.g.*, Kemp *et al.*, 1986; Tikkanen *et al.*, 2006). Such degree-day models are commonly used to determine the appropriate time for the application of pest control measures (Delahaut, 2003).

Although temperature is likely to influence development rate in most insects simply through its effect on biochemical reaction kinetics, this does not tell the complete story about timing of adult emergence. Emergence typically involves the termination of seasonal diapause—with or without some subsequent development, depending on the overwintering stage of the insect. In insects, termination of diapause may occur in response to an external stimulus (*e.g.*, photoperiod, temperature; Tauber *et al.*, 1986) or, more rarely, to an internal biological clock (Blake, 1959). In overwintering bumble bee queens (*Bombus*), warm temperatures alone are insufficient for diapause termination and emergence (Röseler, 1985). Frequently, diapause can only be broken,

or is broken more readily, if insects have experienced a sufficiently long chilling period (Kimberling & Miller, 1988; Gomi, 1996; Bosch & Kemp, 2003; Bosch & Kemp, 2004). This is analogous to the vernalization requirement of plants, and clearly has a similar adaptive value in preventing inappropriate emergence during warm spells in the autumn or winter. In addition, insect activity is temperature-dependent (de Jong *et al.*, 1996; Willmer & Stone, 2004; Saastamoinen & Hanski, 2008), so that even if development is complete and diapause has been broken, insects are unlikely to be observed if weather is cool and overcast.

The available long-term phenology records also underscore the importance of temperature for insects: spring-time air temperatures are good correlates of the first appearances of insects such as honey bees (Gordo & Sanz, 2006) and butterflies (Sparks & Yates, 1997; Roy & Sparks, 2000; Gordo & Sanz, 2006). One study, at an Arctic site, found date of snowmelt to be a better predictor than temperature of the timing of arthropod appearance in pitfall traps (Høye & Forchhammer, 2008); however, the temperature data used for comparison were monthly means from a nearby weather station, so the predictive value of temperature may have been underestimated.

Unfortunately, phenology of pollinating insects, specifically, is difficult to monitor in an unbiased way. If records come from insects at flowers—where most pollinators are captured—the data cannot be independent of the phenology of the plants. Documenting a temporal mismatch between plants and pollinators is impossible using data collected in this way, unless one makes numerous assumptions (*e.g.*, Memmott *et al.*, 2007). Fortunately, there are laboratory data on one group of pollinators—bees in the genus *Osmia* (Megachilidae), some of which are important pollinators of orchard crops—and these reinforce the conclusion that temperature is a key determinant of phenology. *Osmia* spp. overwinter as adults and emerge in spring, meaning that temperature-dependence of larval development in the previous summer (Bosch & Kemp, 2000; Kemp & Bosch, 2005) is essentially irrelevant to emergence phenology. However, there is also evidence that bees emerge earlier when incubated at warm temperatures (Pitts-Singer *et al.*, 2008; White *et al.*, 2009) and after having experienced a sufficiently long chilling period (Bosch & Kemp, 2003; 2004). Growth-chamber experiments with overwintering *O. cornifrons*, in particular, demonstrate that a certain number of degree-days, rather than a specific temperature threshold, is needed to break diapause (White *et al.*, 2009).

We therefore expect temperature to be of primary importance in regulating phenology of most temperate-zone insects, including pollinators, with other factors playing a secondary role. This is similar to the expectation for plants, although timing of snowmelt may be a more important factor in some environments. However, because temperature requirements for insect development and activity may differ from those of plants, it does not follow that temperature dependence in both taxa would ensure synchrony. Furthermore, laboratory studies typically manipulate a single environmental variable in isolation, and hold that variable constant for the duration of the experiment. This approach is extremely useful for determining appropriate rearing conditions for a managed population, but results from these experiments cannot be used on their own to confidently predict phenology—or synchrony with other taxa—in the field, where temperature variability and other environmental factors can come into play (Bosch & Kemp, 2000; Gullan & Cranston, 2000; Watt & McFarlane, 2002). Field studies are also needed to test the applicability of these results to natural communities.

Objectives of this study

We studied insect phenology in subalpine habitats in the Rocky Mountains of Colorado, USA, where several studies have shown that plant phenology is strongly influenced by timing of snowmelt (Inouye *et al.*, 2002; Dunne *et al.*, 2003; Inouye *et al.*, 2003), but where there is so far little information on the determinants of pollinator phenology. Plants and one group of insects in particular—the cavity-nesting Hymenoptera—seem potentially vulnerable to phenological decoupling. This is because these insects overwinter in trees, above-ground, and therefore seem less likely than plants to be influenced by the date of bare ground. We therefore asked 1) which weather variables are most closely correlated with insect emergence, 2) do the same variables predict timing of plant flowering, and 3) to what extent do plant and insect phenology covary? To answer these questions in the absence of a long-term dataset on insect phenology, we used spatial variation as a proxy for temporal variation in climate. Over three years, at sites along an elevational gradient, we collected data on air temperatures, insect emergence phenology, and flowering phenology. Emergence traps on insect nests allowed us to document pollinator phenology independently of flowering phenology (*cf.* Minckley *et al.*, 1994). We experimentally verified that local environmental conditions, rather than local genotypes, were the main determinants of insect phenology by conducting a reciprocal transplant. We then evaluated

various phenology models for several species of plants and insects to determine whether both groups have similar phenological responses to climatic variation.

Methods

Study system

Many species of flower-visiting wasps and bees nest in pre-existing tunnels in wood and will readily occupy artificial structures (“trap-nests”). Of these insects, only the bees are wholly and directly dependent on flowers for larval provisioning; the wasps are either predators of other arthropods or brood parasites of other cavity-nesters (Table 5.1). However, as adults, all of these cavity-nesting species are flower visitors and use floral nectar as their main source of energy, and both bees and their brood parasites require pollen for larval growth. Trap-nests provide standardized over-wintering conditions and can be monitored for insect emergence in spring.

Study sites

In May of 2007 and 2008, we set out experimental trap-nests at a total of fourteen sites in the West Elk Mountains of central Colorado, USA (Table 5.2). At each site, we deployed trap-nests at a height of approximately 1m from the ground by attaching them to trunks of trees located at the edge of a meadow or clearing. The four sites with the highest occupancy rates in 2007 were used again in 2008; we also added six new sites in 2008. Although the sites span an elevational gradient of only 350 m, they encompass substantial variation in climate and phenology: The highest and lowest sites used in 2007 differed by 43 days in the date at which nests became snow-free in spring 2008, and differed by 2.5°C in June mean temperatures.

Field methods

Trap-nests

Details of trap-nest construction differed slightly between years (Plate 5.1). In 2007, we placed four nest blocks at each site (eight at the reciprocal transplant sites; see below), each block consisting of a 22 cm section of 14 × 14 cm untreated softwood lumber. Blocks were prepared as described by Cane *et al.* (2008), except that we used four different diameters for nest holes (3.2–7.9 mm) and different types of hole lining (paper or cardboard straws, or no lining) in an effort to

Table 5.1. Trap-nesting Hymenoptera investigated in this study. Food source records of wasp larvae are from Krombein (1967) except *G. kirbii* (Townes, 1950). Food sources of bees are from Rust (1974), Michener (1947), Mitchell (1960; 1962), Torchio (1989), Strickler, Scott and Fischer (1996), Sheffield, Kevan and Smith (2003), and personal observations. Note that *Gasteruption*, *Chrysis*, *Omalus*, and *Stelis* are brood parasites; the other species are primary nest occupants. All 15 species consume floral nectar as adults, and all bees consume nectar and pollen as larvae. “×” indicates that the species was sufficiently abundant for inclusion in a particular analysis (RT = reciprocal transplant, NH = nest height experiment, PM = phenology modelling). See Appendix D for the full list of taxa recorded in trap-nests.

Family	Species	Larval food source	RT	NH	PM
Wasps:					
Gasteruptionidae	<i>Gasteruption kirbii</i>	Larvae of trap-nesting bees or sphecoid wasps	×		×
Chrysididae	<i>Chrysis coerulans</i>	Prey of <i>Ancistrocerus</i> or <i>Symmorphus</i> spp.	×	×	×
	<i>Omalus aeneus</i>	Prey of <i>Passaloecus cuspidatus</i>	×		
Vespidae	<i>Ancistrocerus</i> <i>albophaleratus</i>	Moth caterpillars			×
	<i>Symmorphus cristatus</i>	Larvae of chrysomelid beetles	×		×
Sphecidae	<i>Passaloecus cuspidatus</i>	Aphids	×		
	<i>Trypoxylon frigidum</i>	Spiders			
Bees:					
Colletidae	<i>Hylaeus annulatus</i>	Multiple plant species	×		×
Megachilidae	<i>Hoplitis fulgida</i>	Multiple species, mainly Rosaceae	×		×
	<i>Osmia coloradensis</i>	Asteraceae		×	
	<i>Osmia iridis</i>	Plant species unknown		×	×
	<i>Osmia lignaria</i>	Multiple plant species	×		
	<i>Osmia tersula</i>	Multiple plant species			×
	<i>Megachile relativa</i>	Multiple species, mainly Asteraceae		×	
	<i>Stelis montana</i>	Nest stores of <i>Osmia</i> spp.			

Table 5.2. Sites used in the study, showing year(s) in which each site was used.

Site name	Latitude (N)	Longitude (W)	Elevation (m)	2007-08	2008-09	2009-10
Cement Creek	38° 49' 17.8"	106° 52' 9.8"	2682		×	×
Brush Creek 1 ¹	38° 51' 39.8"	106° 55' 10.2"	2729	×		
Brush Creek 2	38° 51' 47.9"	106° 54' 54.0"	2743		×	×
Mt. Crested Butte	38° 53' 21.1"	106° 57' 44.3"	2889		×	
Rosy Point	38° 55' 57.6"	106° 58' 12.6"	2900	×		
South Gothic	38° 57' 16.6"	106° 59' 6.7"	2926		×	
Research Meadow	38° 57' 21.5"	106° 58' 55.7"	2929	×		
Marmot Meadow	38° 58' 40.4"	106° 59' 57.1"	2938		×	×
Kebler Clearing	38° 51' 30.9"	107° 3' 37.7"	2958	×	×	×
Splain's Gulch	38° 51' 24.2"	107° 4' 28.4"	2967	×	×	×
401 Trail ²	38° 57' 41.7"	106° 59' 5.6"	2970	×	×	×
Snodgrass	38° 55' 15.6"	106° 58' 16.3"	2999		×	×
Irwin Junction	38° 51' 21.7"	107° 5' 22.6"	3009	×		
Kebler Pass ¹	38° 51' 5.0"	107° 6' 0.0"	3034	×	×	×

¹Sites used in reciprocal transplant experiment.²Site used for nest height study.



Plate 1. Examples of trap-nests used in (a) 2007–2008 and (b) 2008–2010.

attract a range of insect species. Fifty holes, 14 cm deep, were drilled in each block. Asphalt roofing shingles provided some protection from rain. In 2008, for each individual nest, we used a single 14 cm long section of 3.8×3.8 cm lumber with a hole drilled lengthwise through the middle. Ten of these nests were bound into a single unit (“nest block”), to which an overhanging hardboard roof was attached. At each site, ten of these nest blocks (20 at the “401 Trail” site; see below) were attached to trees, as in 2007. On average, we used larger nest holes in 2008: Hole diameters ranged from 6.4 mm to 9.5 mm, again with different types of paper liners.

In mid-August 2007, when most nesting activity was finished, emergence traps were attached to all nest blocks. Each trap consisted of a durable mesh panel supported by a wooden frame with a plugged access hole. A LogTag air-temperature logger (MicroDAQ.com, Contoocook, NH, USA) was attached to the underside of one nest at each site to record temperatures hourly throughout the winter and following summer.

For the trapnests established in 2008, we attached emergence traps the following May. These consisted of transparent vials attached to the front of each occupied nest hole, so that insects could emerge into the vials. A HOBO pendant temperature/light data logger (Onset Computer Corp., Bourne, MA, USA) was attached to the underside of one centrally located nest block at each site (two at the 401 Trail site; see below) and took hourly readings from July 2008 to July 2010 at all but two sites. At the remaining two sites, data loggers were removed in September 2009 because of damage to the nest blocks.

Reciprocal transplant experiment

In 2007, we established twice as many trap-nests at the highest- and lowest-elevation sites as at the remaining sites. In late summer, after emergence traps had been attached, four of eight nest blocks at the highest site were switched with four blocks at the lowest site, so that insects occupying these nests would experience overwintering and springtime conditions at the transplant site. Because nests of different insect species were unevenly distributed among nest blocks, we assigned blocks non-randomly to the transplant and control treatments in an effort to achieve a balanced design for as many species as possible.

Nest height study

In 2008, at the 401 Trail site, we also deployed a double set of trap-nests. The objective of this study was to decouple the effects of snowmelt date and spring–summer air temperature on insect phenology by having nest blocks experience differing durations of snow cover in an otherwise similar microenvironment. To do this, we used a paired design: Lower blocks were attached at 50 cm above ground level, and upper blocks were attached to the same 10 trees at 125 cm.

(Maximum snowpack commonly exceeds 150 cm in this area; b. barr, pers. comm.) One lower and one upper block were outfitted with HOBO data loggers.

Nest monitoring and flower censuses

Starting in early May of 2008 and 2009, and continuing until late July, trap-nests were monitored every 2–4 days for emerging insects. Nests at all sites were monitored less frequently (1–2 times per week) during August 2008 and 2009. We also monitored nests approximately weekly between early May and mid-July 2010 because we observed that many nesting *Osmia* had remained in diapause through 2009. In 2008 and 2009, insects that were alive when we checked traps were assumed to have emerged on that date; those that were already dead were assigned an emergence date two days earlier. In 2010, dead insects were assumed to have emerged four days earlier, because of the reduced sampling frequency. Bees and wasps were preserved and identified to species, if possible, using keys in Krombein (1938), Sandhouse (1940), and Bohart & Menke (1976) for Sphecidae; Bohart & Kimsey (1982) for Chrysididae; Bequaert (1943), Carpenter & Cumming (1985), Cumming (1989), and Buck *et al.* (2008) for Eumeninae (Vespididae); Townes (1950) for Gasteruptiidae; Sandhouse (1939) and Michener (1943) for Megachilidae; and Snelling (1970) for *Hylaeus* (Colletidae). Voucher specimens are deposited in the Royal Alberta Museum (Edmonton, AB, Canada), the USDA–ARS Pollinating Insect Research Lab (Logan, UT, USA), and the RMBL insect collection.

On each day from May to late July 2008 and 2009 that we checked a site for emerging insects, we also monitored flowering plants. We established three or four 100 m permanent belt transects at each site, radiating out from the trap-nests in approximately the four cardinal directions. Some transects were truncated if they encountered a barrier such as a road, cliff edge, or dense forest. The choice of a 100 m radius is somewhat arbitrary, and would almost certainly underestimate resources available to newly emerged insects: Typical natal dispersal distances are unknown, but

Osmia-sized solitary bees can fly distances of several hundred metres (Gathmann & Tschardt, 2002; Greenleaf *et al.*, 2007). However, preferred foraging ranges are certainly less than this in several species (see, *e.g.*, Peterson & Roitberg, 2006; Greenleaf *et al.*, 2007; Zurbuchen *et al.*, 2010). In any case, as the purpose of the transects was to estimate phenological progression of the flowering plant community (*i.e.*, the timing of peak flowering for various species) rather than to quantify resource availability, a 100 m radius provides a reasonable index. Transect widths ranged from 10 to 200 cm, depending on the size and population density of the species being counted. For most plant species, we counted the number of open flowers, or capitula (Asteraceae), found on the transects. For species with many flowers per plant (*Mertensia* spp. [Boraginaceae], *Viola praemorsa* [Violaceae]), we instead counted the number of whole plants that had at least one open flower. Plant taxonomy follows Hartman and Nelson (2001). *Mertensia fusiformis* and *M. brevistyla* are morphologically and phenologically very similar (and frequently synonymized; Hartman and Nelson 2001), so we treated these as a single taxon here, called “*M. fusiformis*” for convenience (*M. brevistyla* occurred only at the Kebler Pass site).

Data analysis

Estimating snow cover from weather data

We assumed that a recorded daily temperature range of $< 5^{\circ}\text{C}$ indicated insulation of nest blocks by snow. In the 2008–2009 and 2009–2010 winters, when data-loggers recorded light as well as temperature, we were able to confirm that these periods of low temperature variability corresponded with periods of low daily maximum light intensities (< 1000 lux, compared to typical maxima of $\sim 30\,000$ lux on sunny summer days). Duration of snow cover was therefore estimated as the interval between the first and last days with daily ranges $< 5^{\circ}\text{C}$ (excluding brief, isolated periods of low temperature variability).

Reciprocal transplant experiment

A balanced three-way ANOVA, with species, site of emergence, and site of origin as factors predicting emergence date, would be desirable but was not possible because each species was absent or rare in at least one of the four treatment combinations. We therefore treated species, rather than individual insects, as replicates, and tested for an effect of site of emergence (high or low) and site of origin (high or low) using two separate paired *t*-tests on mean emergence dates.

Differences between sites in mean emergence dates were approximately normally distributed. For species represented by at least five individuals per treatment in at least two treatments, we were also able to test for treatment effects on emergence date for each species, using individual bees as replicates. Here, we tested for effects of site of origin and site of emergence separately, using Kruskal–Wallis (Mann–Whitney) tests because emergence dates were non-normally distributed.

Nest height study

Species that were represented by at least five individuals in both the upper (1.25 m) and lower (0.5 m) nests were used for analysis. Emergence dates were not normally distributed, so we tested the effect of nest height using Kruskal–Wallis tests.

Estimating temperature effects on emergence and flowering

We wished to determine the most likely phenology model for each plant and insect species that was well represented in our dataset. Preliminary analyses showed that peak dates of flowering and insect emergence at different sites in 2008 were predicted well by degree-days above a base temperature between 0°C and 10°C in the preceding months; for most species, degree-days were better predictors than snowmelt date, various candidate threshold air temperatures, or mean temperatures in the preceding months. Consequently, for the remainder of the analysis, we focused on developing degree-day models for each species and seeing whether plants and insects responded similarly.

We focused on the eight insect species (Table 5.1) for which we had collected at least 50 individuals across all sites and years (range = 51–540) and that were present at a minimum of four sites. All but two (*Hylaeus annulatus* and *Osmia tersula*) were adequately represented in at least two years of the study. The six focal plant species were those which were abundant at a majority of sites and for which our monitoring had captured most or all of the flowering period at each site. For the two focal species for which we had missed the end of flowering (*Linum lewisii* [Linaceae] and *Helianthella quinquenervis* [Asteraceae]), we fitted sine curves to the existing data (following Malo, 2002) to allow us to estimate flower densities on later (hypothetical) sampling dates. In all cases, these estimates indicated that our sampling had captured more than 95% of all flowering. Using the actual or estimated flower counts, we calculated the cumulative proportion of flowering for a given species by a given date as the sum of all flower counts of that

species prior to and including that sampling date, divided by the sum of all counts of that species over all sampling dates (in a fixed sampling area).

For each sampling date or estimated emergence date, we calculated accumulated degree-days until that date using the hourly air-temperature data from each site (provided as online supplementary material). We calculated degree-days since 1 January (DD) using a range of base temperatures (0–15°C in 1°C increments), because the lower threshold temperature for development or activity was unknown for these taxa and had to be estimated from the data. For each species, we fit generalized linear mixed models, with binomial error and logit link, to the cumulative proportion of emergence or flowering that had occurred by a given date. Initially, we used degree-days above a particular base temperature, and site (a random factor) nested within year (fixed), as predictor variables. However, the year effect could not be tested for all species (some were recorded in only one year), and it was non-significant in many, but not all, of the others. We therefore created a single, composite, random variable, “site-year”, to encompass all site + year combinations. Similarly, we allowed random variation in regression intercepts for each site-year, but slopes were fixed because inclusion of a random slope term did not significantly improve model fit for most species. Logistic models are commonly used for phenology modelling (*e.g.*, Kemp & Onsager, 1986; Manel & Debouzie, 1997; Meagher & Delph, 2001; Régnière *et al.*, 2007), and although they will not provide the best fit in cases where phenological events are not normally distributed (Brown & Mayer, 1988), we found that they generally provided a good fit to our data (see Figs. 5.1–5.2). Data transformations did not improve model fit, so we used untransformed data. We used the `lme4` package in R (R Development Core Team, 2007) to fit logistic models, using each possible base temperature for DD accumulation. Because models did not differ in the number of parameters, models were compared simply on the basis of their log-likelihoods.

Because we found highly significant effects of site-year in these models ($P < 0.0001$ for all species, according to likelihood-ratio tests against models with site-year omitted), we attempted to explain these effects as a consequence of among-site and between-year variation in the amount of chilling. We defined “chill days” as hours between 1 October and 31 March in which local temperature was below 5°C, divided by 24. (We tested alternate definitions, such as higher or lower threshold temperatures and greater ranges of dates, and found that these gave qualitatively

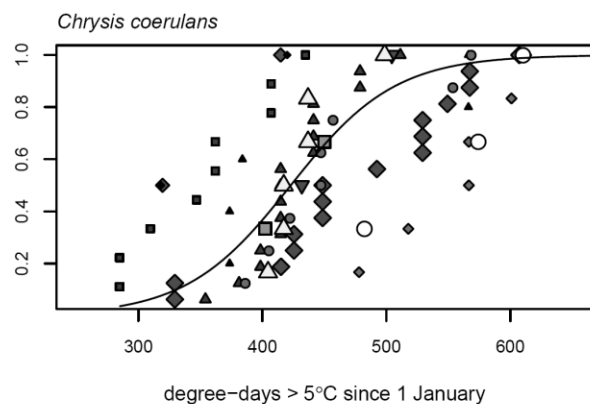
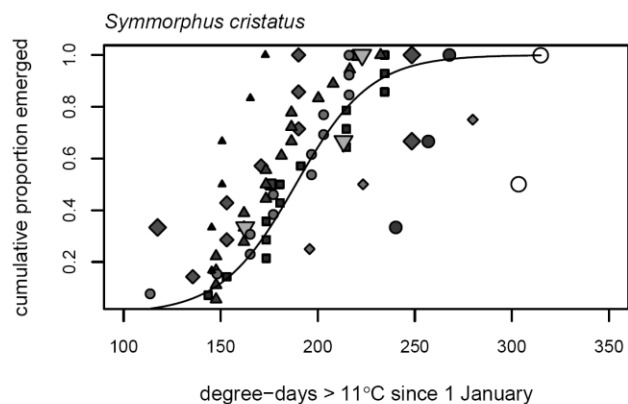
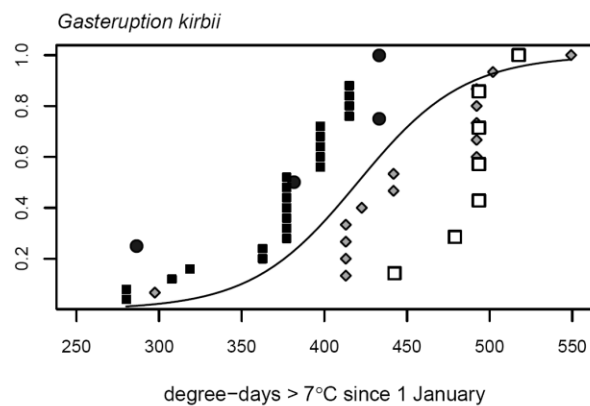
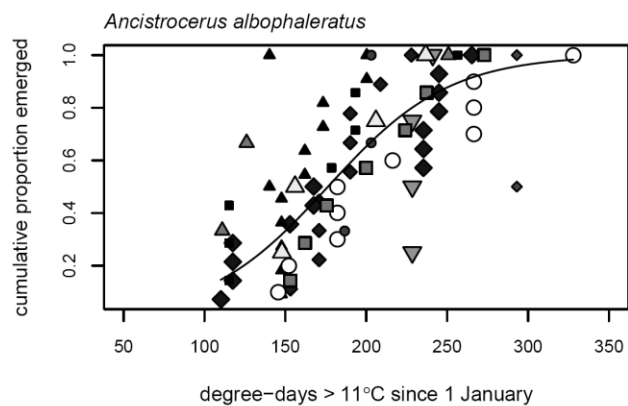
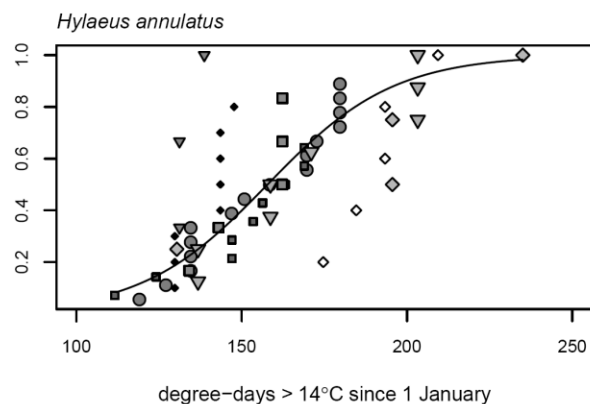
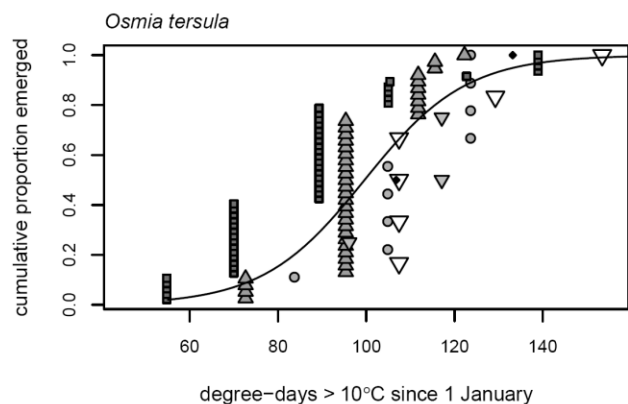
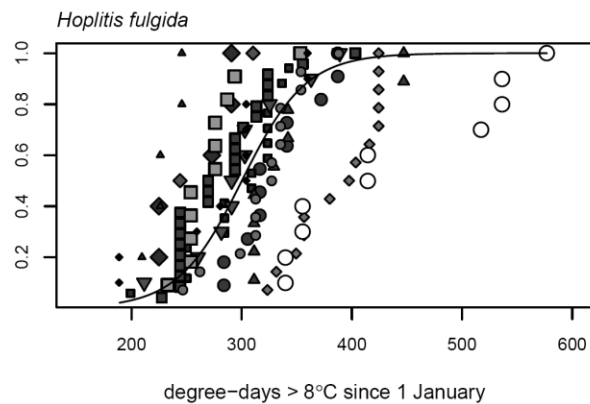
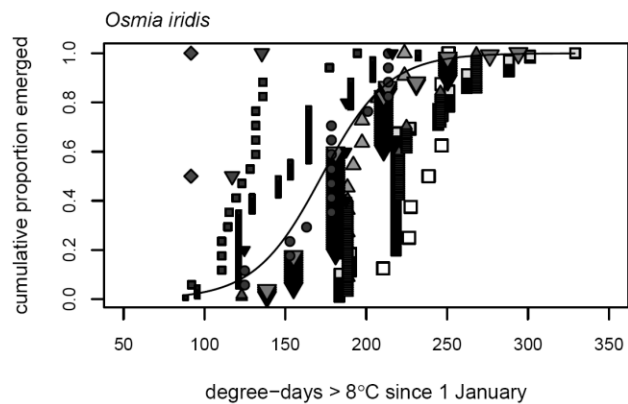


Figure 5.1. (*opposite page*) Emergence phenology of eight species of trap-nesting Hymenoptera at sites along an elevational gradient. The cumulative proportion of emergence having occurred on each sampling date at each site is plotted against degree-days accumulated up to that date. Note that different base temperatures are used for each species; these are the base temperatures that provide the best model fits across all sites and both years. Different site-year combinations are indicated by different symbols. Symbol shading corresponds to the number of chill days (see text) experienced at each site in the preceding winter (lighter shading = fewer chill days); sites experiencing less chilling tend to be found further to the right in each plot, indicating that they require more heat accumulation for emergence. Lines show the best-fit logistic regressions for each species over all site-years combined (regressions for individual site-years are omitted for clarity).

similar, though typically weaker, results. Low, but not sub-zero, temperatures are required for vernalization in *Arabidopsis thaliana* [Kim *et al.*, 2009] and are regularly used for overwintering *Osmia* [*e.g.*, Kemp & Bosch, 2005; White *et al.*, 2009].) We then tested, using linear regression, whether the site-year-specific intercepts of the best-fit logistic regressions could be predicted by chill days. As an alternative, we also tested whether start dates for DD accumulation later than 1 January improved model fit. Later start dates have the effect of discounting any heating that occurs early in the year, effectively penalizing lower-elevation or earlier-warming sites for their head start in DD accumulation, and producing an effect similar to the incorporation of chill days in the model. Specifically, we tested start dates between 22 March and 10 June (or the latest possible date prior to the onset of each species' emergence or flowering), in 10-day increments. We chose 22 March because little heating occurs before this date at any site.

Decoupling of plants and pollinators

Finally, to evaluate whether differences in the temperature responses of bees and plants could result in their phenologies coinciding in some sites or years, but not others, we plotted median emergence dates of *H. fulgida*, the best-represented bee species in our 2008–2009 samples ($N = 142$), against median flowering dates of two plant species it is known to visit, for each site-year. Points falling along the 1:1 line in these plots would indicate site-years with perfect synchrony between insect emergence and plant flowering. Points lying above the line represent site-years in which flowering was past its peak by the time of peak insect emergence; such a scenario would

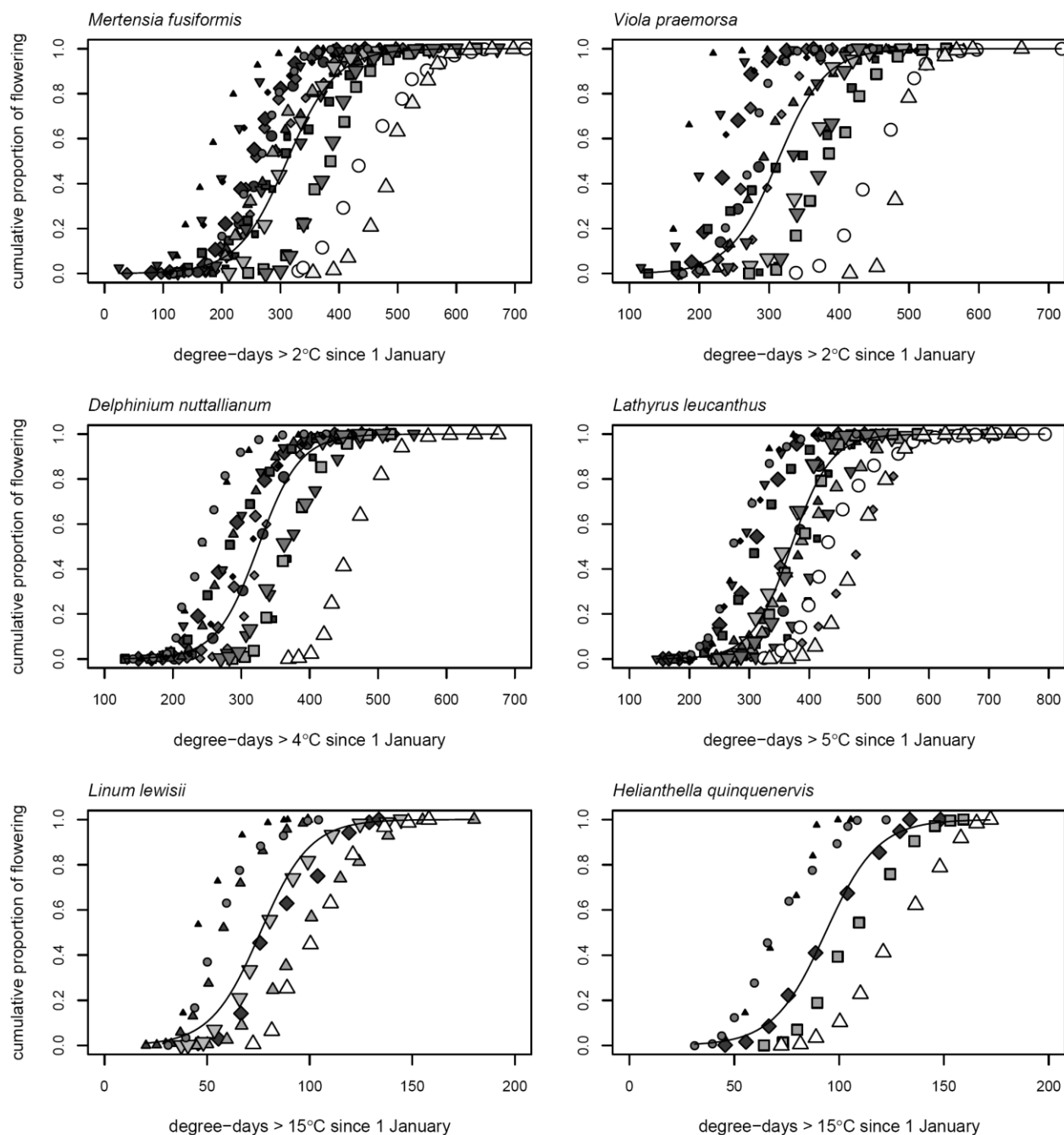


Figure 5.2. Flowering phenology of six plant species at sites along an elevational gradient. Lines and shading as in Fig. 5.1.

presumably make that resource largely unavailable to the insects. In contrast, points below the line represent site-years in which insect emergence precedes flowering; if the precedence is not too great (*i.e.*, does not exceed a bee's lifespan or its ability to survive without food), these floral resources are likely to be available to foraging bees. *Lathyrus lanszwertii* var. *leucanthus* (henceforth *Lathyrus*; Fabaceae) is the plant species in our dataset with a phenology most similar to that of *H. fulgida*. However, *Potentilla hippiana* × *gracilis* (henceforth *Potentilla*; Rosaceae) is a species on which *H. fulgida* females are frequently seen collecting pollen, and it may be a more important resource for this bee. Both plant species are visited by other insects; neither is dependent on *H. fulgida* for cross-pollination. We did not include *Potentilla* in previous analyses, because our sampling did not capture its full flowering period at most sites, and it has an indeterminate and frequently bimodal flowering curve that defied all attempts to fit parametric models. (This may be because this taxon encompasses multiple hybrid strains, with slightly different phenologies, that could not be distinguished in the field.) Here, for illustrative purposes, we treated the data as though we had captured the full flowering curve at each site. It should be noted that doing so biases estimates of peak flowering toward earlier dates, particularly at late-flowering sites; however, it does not affect the earlier part of the flowering curve, with which *H. fulgida* emergence coincides.

Results

In total, 412 insects representing 25 species of Hymenoptera, excluding Ichneumonoidea, emerged from experimental trapnests during the 2008 season; 731 insects (27 species, of which 18 were also present in 2008) emerged in 2009; and 963 insects (8 species) had a two-year life cycle, emerging in 2010 (Appendix D). *Osmia iridis* and *O. tersula* were present in all three years, indicating that these species are parsivoltine in our study area (*i.e.*, capable of a 1- or 2-year life cycle; Torchio & Tepedino, 1982); but the latter was represented by only a single individual in 2009. Most species were rare (< 50 individuals across all three years), but those that were sufficiently numerous for analysis are listed in Table 5.1.

Reciprocal transplant experiment

Nine insect species occurred in at least two of the four reciprocal transplant treatments (two sites of origin × two transplant sites). Considering all of these together, there was no indication of a site-of-origin effect on emergence date (paired *t*-test, $t_5 = 0.42$, $P = 0.69$; Fig. 5.3). However,

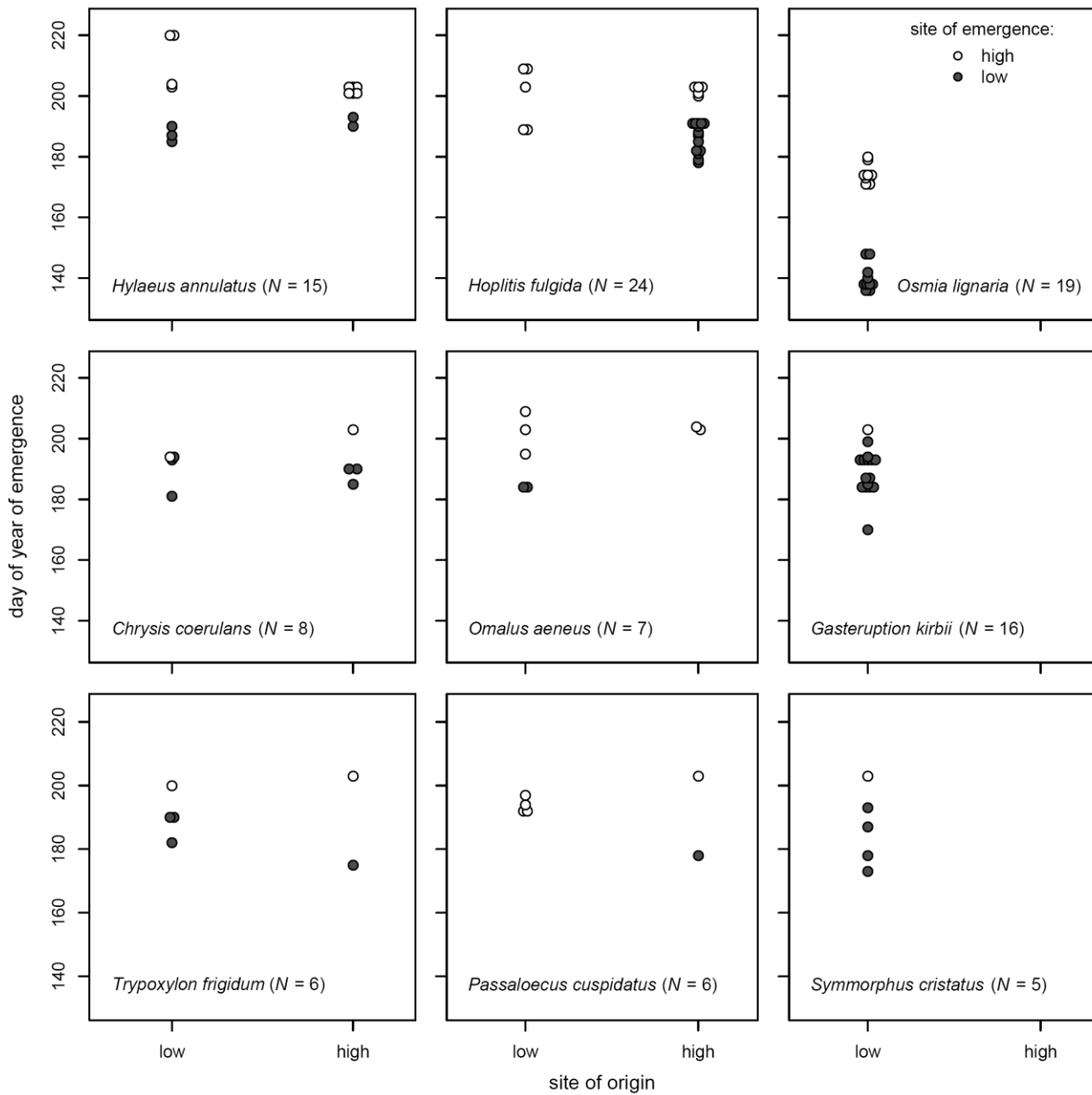


Figure 5.3. Emergence dates for nine species of trap-nesting Hymenoptera occurring in the reciprocal transplant experiment, as a function of site of origin and site of emergence. Each point represents one insect. Filled points represent insects emerging at the low-elevation site; open points represent the high-elevation site. Points have been jittered for clarity. Sample sizes are indicated in each panel.

insects overwintering at the high-elevation site consistently emerged later than those at the low-elevation site (paired t -test, $t_8 = 8.20$, $P < 0.0001$; Fig. 5.3). To check that the significance of the latter test was not solely due to its larger sample size, we re-ran the test with all possible subsets of $N = 6$ species; the highest P -value obtained was 0.0034, suggesting that the result is robust.

These overall patterns were consistent with those obtained from the two species (both bees) that we were able to test individually: *Osmia lignaria* occurred naturally only at the low-elevation site, where it emerged in mid-late May, but it emerged from transplanted nests at the high site, on average, 34 days later (Kruskal–Wallis $\chi^2 = 13.7$, $P = 0.00021$, $N = 10 + 9$). *Hoplitis fulgida* individuals originating at the high site also emerged 16 days later at that site than when transplanted to the low site (Kruskal–Wallis $\chi^2 = 10.7$, $P = 0.0011$, $N = 14 + 5$); conversely, bees over-wintering at the high site emerged at approximately the same time (18 or 20 July, on average), regardless of site of origin (Kruskal–Wallis $\chi^2 = 0.047$, $P = 0.83$, $N = 5 + 5$). (In comparison, snow melted from high-elevation nests 43 days later than from low-elevation nests.) Thus, overall, we find no support for the hypothesis of local adaptation in insect phenology, and strong evidence of environmental determination of emergence time.

Nest height study

The upper (1.25 m) nests were not snow-covered during either the 2008–2009 or the 2009–2010 winters, whereas the lower (0.5 m) nests were snow-covered, except for brief thaws, from 16 December 2008 to 8 April 2009 and from 22 January to 8 March 2010. Upper and lower nests experienced nearly identical temperatures after snowmelt, but earlier in the spring, upper nests experienced much greater temperature variation, and, consequently, had accumulated many more heating units between 1 January and 1 May (*e.g.*, degree-days $> 5^\circ\text{C}$: 213 vs. 38 in 2009, and 178 vs. 35 in 2010).

Five insect species occurred in both upper and lower nests in sufficient numbers for analysis (Table 5.1). For each species, we analysed the sexes separately, either because one sex did not occur at one or both of the nest heights, or because strong covariances between sex ratio and nest position would have biased results (males emerge before females in all these species). For the one species (*Osmia iridis*) that emerged in both years of the study, there was no difference in phenology between years, so data from both years were combined for analysis.

Emergence dates of *Megachile relativa* and *Stelis montana* were unaffected by nest height ($P > 0.3$); *M. relativa* individuals even tended to emerge later from the upper nests. For the two other bee species (*O. coloradensis* and male and female *O. iridis*) and the wasp *Chrysis coeruleans*, timing of emergence was significantly affected by nest position (Kruskal–Wallis $\chi^2 > 4.0$, $df = 1$, $P < 0.05$; Fig. 5.4). A significant nest-position effect remains for *C. coeruleans* and *O. iridis* even after applying a Bonferroni-corrected α of 0.01; note, however, that it is the magnitude of the effect, rather than its significance, that is of most interest. Individuals of both *Osmia* species and the wasp emerged only 2–12 days later, on average, from the lower nests than the upper ones. Even the 12-day delay seen in *C. coeruleans*, the species showing the largest effect of nest height, translates into an increment of only 105 DD $> 5^\circ\text{C}$ —not enough to make up the difference in heat accumulation between 1 January and 1 May.

Temperature effects on emergence and flowering

Regressions of cumulative emergence or flowering against accumulated degree-days were well fit by logistic functions specific to each site-year for all species (Figs. 5.1–5.2). Assuming a 1 January start date for degree-day accumulation, the best-fit models for the eight insect species used degree-days above a base temperature between 5°C and 14°C , depending on the species (Figs. 5.1, 5.5). For most species, these most-likely base-temperature estimates are robust, within 1°C , to the decision not to include site-specific slopes in the regression model. For two species, however (*Chrysis coeruleans* and *Osmia iridis*), adding random slope estimates produces significantly more likely models with higher base-temperature estimates (10°C and 11°C , respectively, instead of 5°C and 8°C).

For four out of six plant species investigated, the best model fits were obtained for base temperatures between 2°C and 5°C , again assuming a 1 January start date for degree-day accumulation (Figs. 5.2, 5.6). For the remaining two (*L. lewisii* and *H. quinquenervis*, the two latest-flowering species we considered), model support increased with increasing base temperature at least up to 15°C ; however, there was little difference in log-likelihood (< 3) over the full 15°C range, indicating only weak relative support for a model with such a high base temperature (Fig. 5.6). For all six species, the base temperature estimate is robust within 1°C to the exclusion of site-specific slopes.

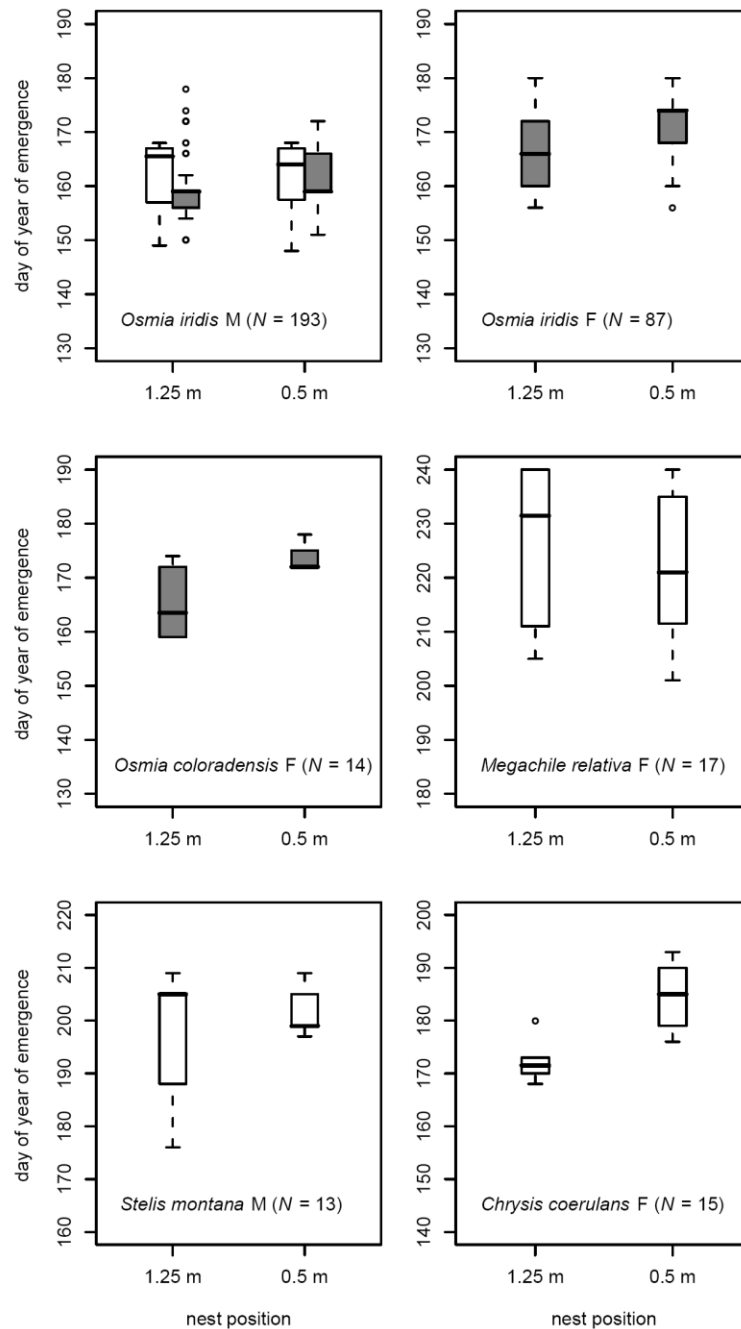
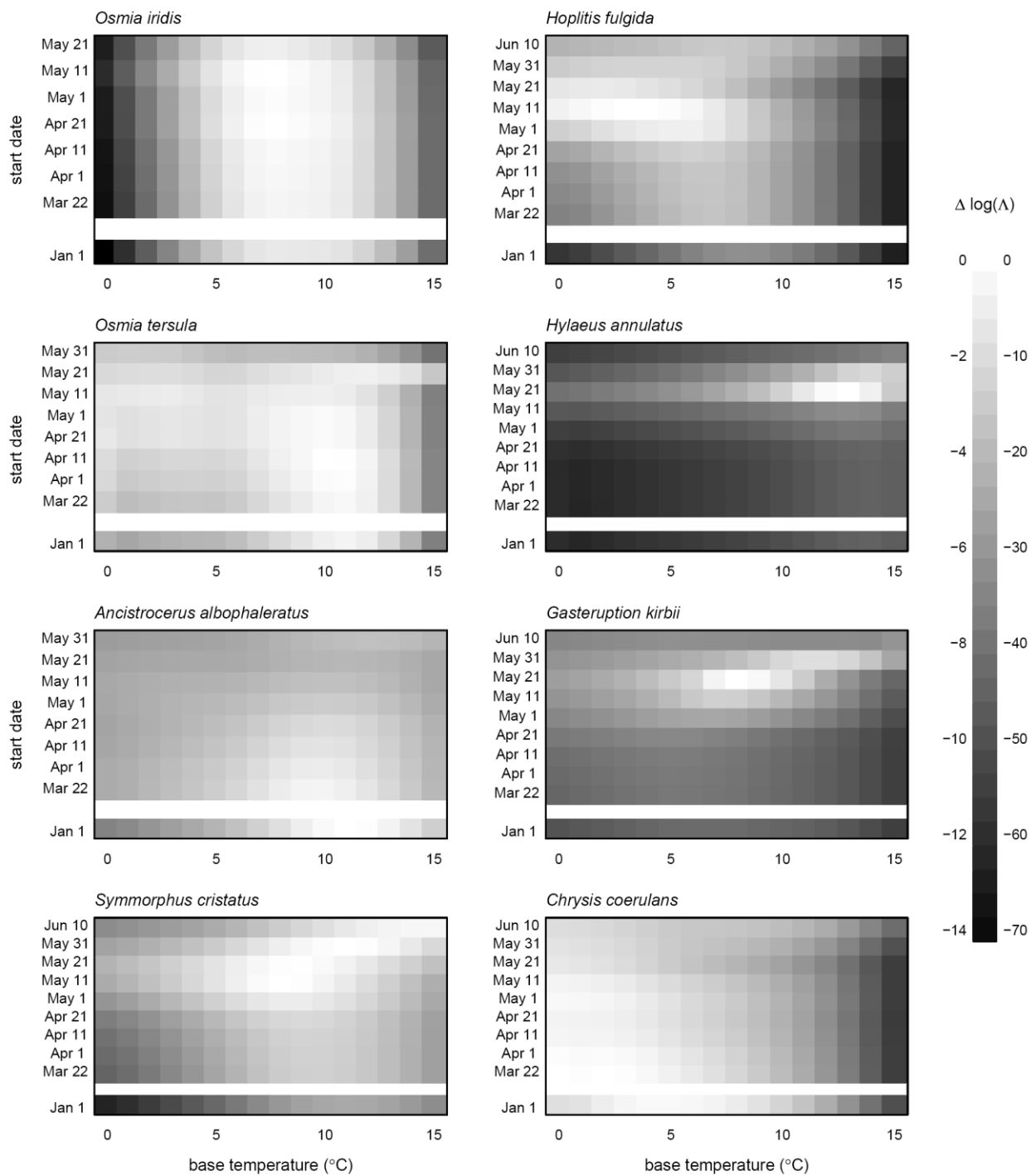


Figure 5.4. Tukey box-plots showing effect of nest position (metres above ground level) on emergence phenology of five species of trap-nesting Hymenoptera. Shaded boxes represent insects emerging in 2010; unshaded boxes represent 2009 emergence. Nests at 0.5 m were snow covered each winter; those at 1.25 m were not. Sample sizes are indicated in each panel. “M” = males; “F” = females. Nest position had a significant effect on emergence date in *O. coloradensis*, *O. iridis* (both sexes), and *C. coerulans*. Boxes show medians and interquartile ranges.

Figure 5.5. (*opposite page*) Log-likelihoods of different phenology models for eight insect species. Each model requires a specified base temperature for degree-day calculation and start date for degree-day accumulation; each shaded square in the figure represents the log-likelihood of a single model and therefore a single combination of base temperature and start date. All combinations of base temperatures between 0 and 15°C (in 1°C increments) and start dates between 22 March and 10 June (or as late as possible, in 10-day increments), plus 1 January, were used for fitting logistic regressions to emergence data (as in Fig. 5.1). Squares are shaded according to the difference in log-likelihood between that model and the most likely model for that species. The square representing the most likely model is completely unshaded; darker shading indicates worse fit. The scale on the right translates differences in shading to differences in log-likelihoods. Values to the right of the scale apply to *Osmia iridis*, which showed greater differentiation among models and therefore a greater range of log-likelihoods; values to the left of the scale apply to all other species. All models use the same number of parameters, so model fits can be assessed by comparison of log-likelihoods, with a difference of much greater than 1 indicating substantially less support for the inferior model (Burnham and Anderson 2002). The fact that models using start dates later than 1 January tend to have greater support suggests that high temperatures occurring early in the season are less effective at hastening development.

Although models based on degree-days accumulated from 1 January fit well for individual site-years, there was substantial remaining variation among sites in degree-days required for 50% emergence or flowering (*i.e.*, the site-year intercept term remains highly significant for all species). Specifically, organisms at lower-elevation or earlier-melting sites tended to require more degree-days than those at higher, later-melting sites. Indeed, for most species, the number of “chill days” experienced in a given site-year was a good predictor of the position of the phenology curve (*i.e.*, the regression intercept): more chilling tends to shift the curve to the left (Figs. 5.1–5.2). This was true of all six plants ($R^2 = 0.42\text{--}0.87$, $P < 0.05$, $N = 5\text{--}18$ site-years), three bee species ($R^2 = 0.42\text{--}0.54$, $P = 0.05$, $N = 8\text{--}13$), and *Gasteruption kirbii* ($R^2 = 0.97$, $P = 0.014$, $N = 4$). For the remaining four insect species, the trend was in the same direction but non-significant ($R^2 = 0.15\text{--}0.30$, $P = 0.079\text{--}0.44$, $N = 6\text{--}12$). Similarly, for all plants and most insects, model fits were greatly improved by the use of start dates for DD accumulation later than 1 January (Figs. 5.5–5.6). This suggests that high temperatures occurring early in the season are ineffective at hastening development in most species. The best models incorporating later start



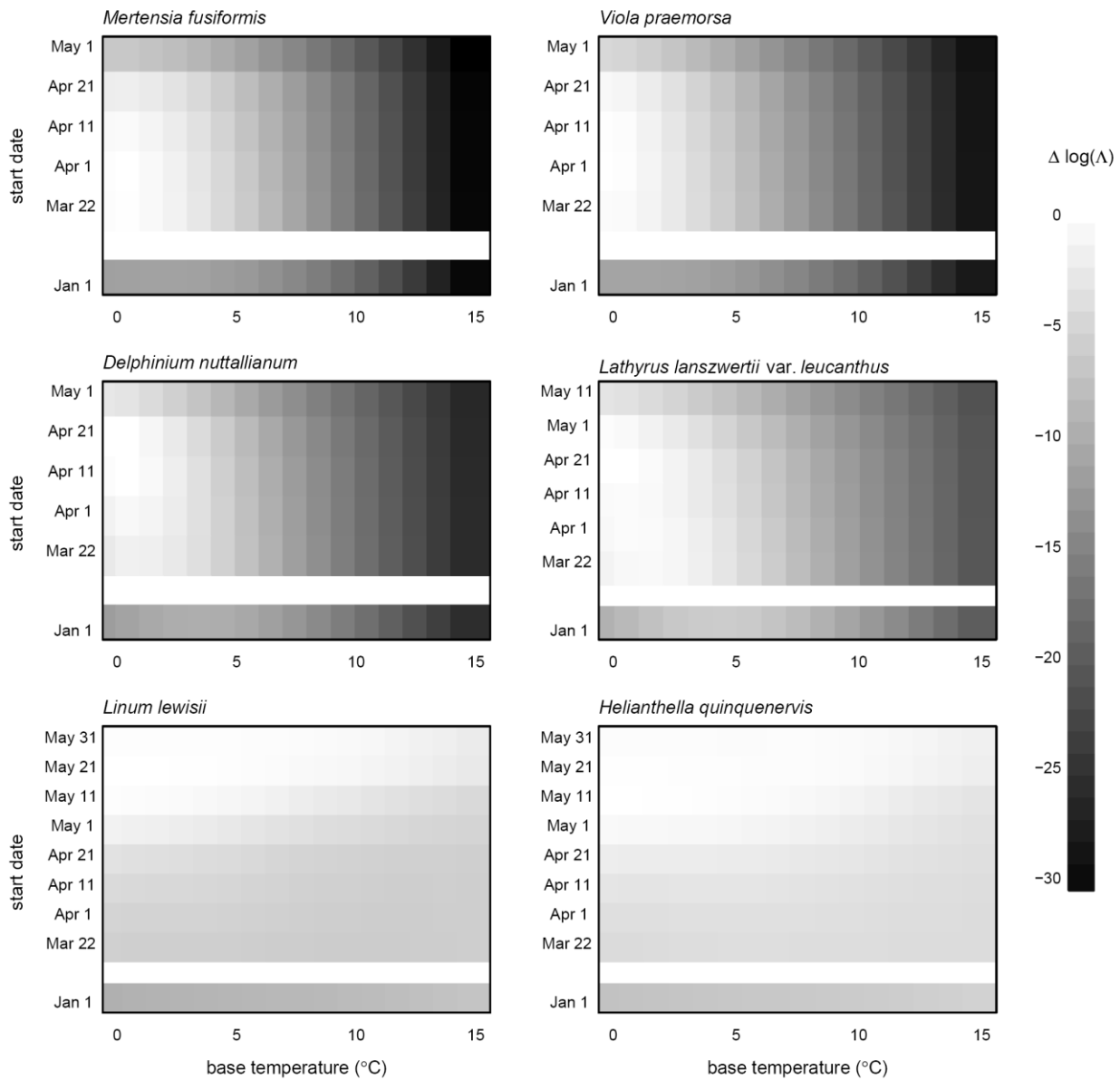


Figure 5.6. Log-likelihoods of phenology models for six plant species. See Fig. 5.5 caption for explanation.

dates explained, on average, 82% of the deviance for each species (range: 62–95%). For several species, using later start dates also changed the estimate of the best base temperature, shifting estimates downward in all six plant species and in a majority of the insects (Figs. 5.5–5.6). The pattern of generally higher base temperatures for insects, compared to plants, remains.

Decoupling of plants and pollinators

Lathyrus and *H. fulgida* showed broad overlap in phenology at most sites in 2008 (Fig. 5.7), in spite of the two species showing modest differences in temperature responsiveness in the previous analysis. However, they showed, at best, only slight overlap at any site in 2009, such that, at all sites, *Lathyrus* had finished or almost finished flowering by the time of peak *H. fulgida* emergence (Fig. 5.7). This was due, in part, to generally earlier flowering by this species at warmer, low-elevation sites in 2009 compared to 2008, without a corresponding advance in phenology of the bees.

In contrast, emergence phenology of *H. fulgida* corresponded reasonably well with that of *Potentilla* in both study years (Fig. 5.8). At one site in 2009, peak bee emergence did not occur until late in the *Potentilla* flowering period; but for most other site-years, emergence matched or preceded peak flowering by several days, a sequence that should ensure that pollen is locally available to female bees while they are provisioning nests. Note that this qualitative result would be unchanged even if we assumed that our median flowering dates for this species are underestimated at the later-flowering sites.

Discussion

Environmental determinants of phenology

Insects in our study showed no sign of local adaptation in timing of emergence. Although sample sizes were small in the reciprocal transplant experiment, we were able to detect a strong effect of local conditions at the emergence site on emergence time, but no effect of site of origin, suggesting that emergence time is purely environmentally determined. Interestingly, we detected no tendency for insects from the high-elevation site to develop more rapidly than low-elevation insects at a given emergence site, a pattern that is often observed along latitudinal and elevational gradients (counter-gradient variation; Conover & Schultz, 1995). The geographic scale of our

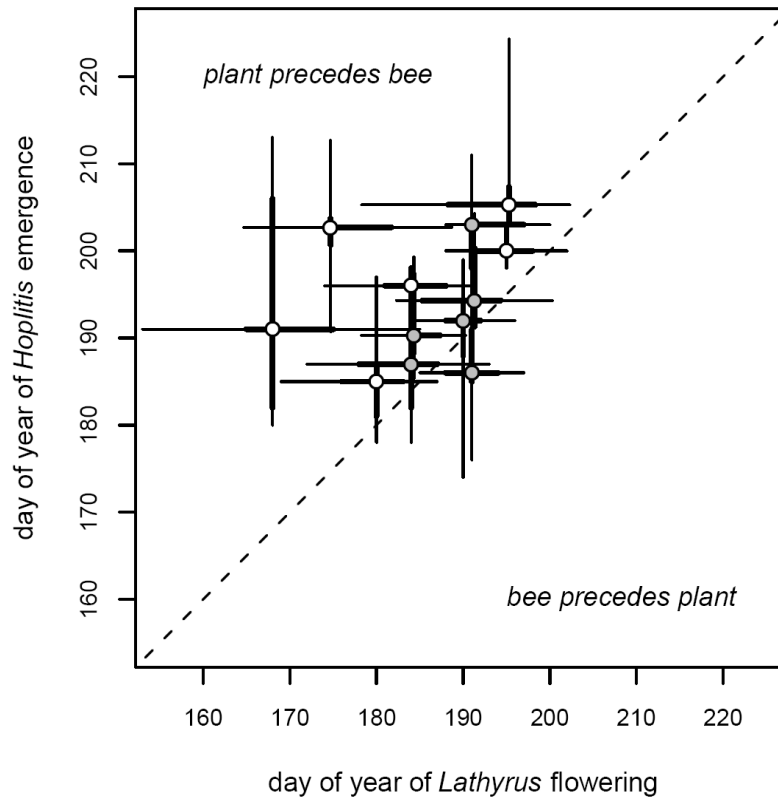


Figure 5.7. Phenology of *Lathyrus* and *H. fulgida*, plotted as median emergence/flowering dates (points), interquartile range (thick lines) and 5–95% range (thin lines) for each site-year. Shaded points represent data from 2008; open points represent 2009. The dashed line is the 1:1 line, representing perfect synchrony between *Lathyrus* flowering and *H. fulgida* emergence. Points above the line indicate that flowering occurred earlier than bee emergence. Some values have been jittered by ± 0.3 d for clarity.

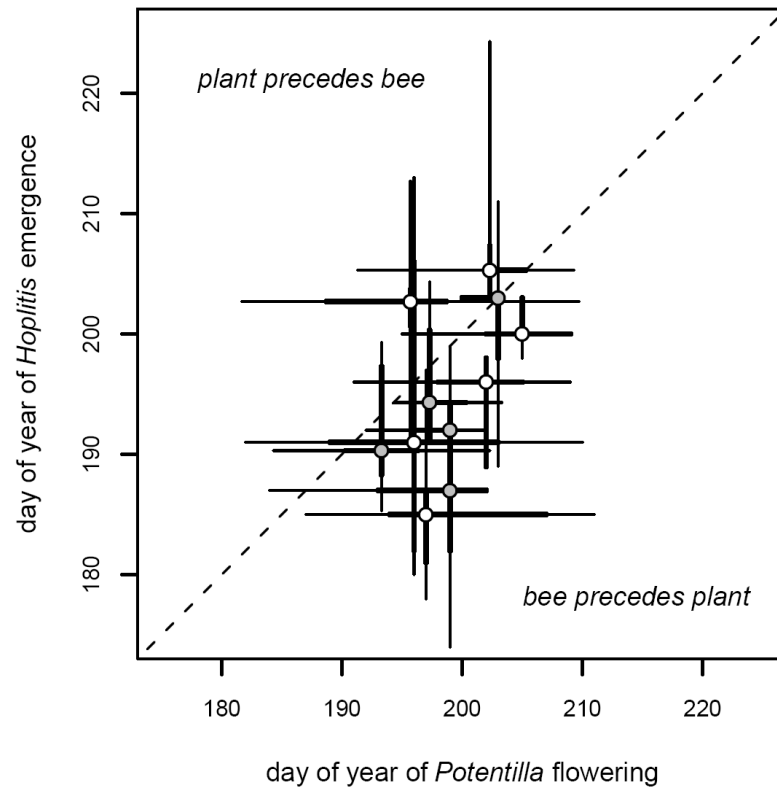


Figure 5.8. Phenology of *Potentilla hippiana* $\times$ *gracilis* and *H. fulgida*. As in Fig. 5.7, except note that plant sampling ended on day 202–203 in 2008 and on day 209–210 in 2009, so the end of flowering was missed at most sites. This tends to bias points (especially those representing later sites, in the upper right of the graph) to the left.

study (~15 km in horizontal distance and 300 m in vertical distance between high and low sites, without major barriers to dispersal) may have been too small to have produced local adaptation. A similar result was reported by Krombein (1967), who noted, anecdotally, that conspecific insects in trap-nests from various localities along a latitudinal gradient in the eastern U.S. emerged at approximately the same time in a common rearing environment in Washington, D.C., likewise suggesting no local adaptation in phenology.

In fact, there may be little selection for compressed phenology in high-elevation insect populations if season length is sufficiently long to complete a generation. Also, if the resources on which insects depend similarly show no differentiation over the elevational gradient in their responses to environmental cues, there may be no advantage to insects in getting a more rapid start to the season at higher sites. We did not test for local adaptation in phenology of the plants in this study, but an earlier common-garden experiment with *Potentilla gracilis* (“*P. pulcherrima*”; Hartman and Nelson 2001) in our study area around the Rocky Mountain Biological Laboratory provides relevant information: That study showed no genetic difference in time to flowering among populations that spanned a greater elevational range (1000 m) than did our study sites; and, furthermore, selection on phenology in that species was similar at all sites, favouring a shorter prefloration interval regardless of elevation (Stinson, 2004). If *P. gracilis* is representative of plants in the area, we would not expect local adaptation in phenology in our study species—although transplant tests with all of them would be required to confirm this.

Given that, in general, phenology of insects and plants is primarily determined by the local environment, which aspects of the environment are most important? Photoperiod can be ruled out as being the sole cue, since it does not covary with season length along an elevational gradient. Snowmelt must also not be a critical factor, at least for insects: All trap-nests were snow-covered in the heavy winter of 2007–2008, but this was not the case in the next two winters, when snowpack under trees did not reach 1 m at most sites. We do not have site-specific data on the date of snowmelt at ground level, so it is possible that this would have provided a good predictor of plant flowering even though the date at which snow melted off the trap-nests (in 2008) generally did not. In the one species (*Mertensia fusiformis*) for which the 1 m snowmelt date was a slightly better predictor of peak flowering than degree-days in the preceding months ($r^2 = 0.90$ vs. 0.88 , $N = 7$ sites), the slope of the relationship was much less than 1 (95% CI: 0.29 – 0.60), indicating that elapsed time between snowmelt and flowering is not

uniform across sites. This lack of uniformity must be accounted for by other factors that vary among sites—if not alleles at phenologically relevant loci (which we have tentatively ruled out), then other components of climate.

For all species we studied, we were able to develop simple degree-day models that explained much of the variation in phenology among sites and years. For most insects, the base temperatures that provided the best fit in these models were higher than for most plants. This accords reasonably well with previous studies that have used base temperatures between 4°C (Kimberling & Miller, 1988) and 18°C (Kemp & Onsager, 1986) to predict insect phenology (see also Campbell *et al.*, 1974; Nealis *et al.*, 1984), and base temperatures of 0–1°C (*e.g.*, Ladinig & Wagner, 2005; Larl & Wagner, 2005; Hülber *et al.*, 2010) for plants. If this is a general pattern, it suggests that increases in average temperatures will hasten phenologies of both insects and plants; but the details of when warming occurs will determine the potential for differential effects on insects and plants. For example, an increase in early-morning temperatures in spring from 0 to 4°C would be expected to promote plant development without affecting insect phenology. It is possible that we observed such an effect in 2009, when June temperatures were cooler than usual in our study area (mean maximum daily temperature in June 2009 = 18.2°C at a weather station near the RMBL, compared with an average of 21.7°C [s.d. = 1.7] for the same period in 2000–2008; b. barr, personal communication). This may explain the lack of correspondence between *Hoplitis fulgida* and *Lathyrus* at most of our study sites in that year: With earlier snowmelt in 2009 than 2008, plants at low-elevation sites were able to start growth earlier in the season, while phenology of the bees remained unchanged (Fig. 5.7).

Although there was a general tendency for our best insect models to require higher base temperatures than did our plant models, there was also substantial variation among insects in both the base temperature estimates and the effects of chilling on emergence phenology. In particular, three wasp species (*Ancistrocerus albophaleratus*, *Symmorphus cristatus*, and *Chrysis coerulans*) showed less effect of chilling on emergence than did plants, most bees, and the bee parasite *Gasteruption kirbii*. This is intriguing, because these wasp species are not closely related but are connected ecologically: *C. coerulans* is a brood parasite of the other two, frequently emerging from the same nest on the same day as individuals of the host species (J. Forrest, personal observation; also Krombein 1967). We might expect *C. coerulans* to have evolved to use similar emergence cues to those used by its hosts. However, phenology models for these

three species are otherwise dissimilar (Fig. 5.5), so the mechanism by which *C. coerulans* maintains (or fails to maintain) synchrony with its hosts requires additional study. One possibility is that the parasite may have a longer flight season than its hosts. *Chrysis coerulans* also parasitizes nests of other eumenine wasps, some of which occurred at our study sites, so the need for synchrony with *A. albophaleratus* and *S. cristatus* is not absolute (although the other eumenines have similar phenologies).

Interspecific variation in responsiveness to chilling may also have played a role in our nest-height experiment. For all five species occurring in that experiment (four bees and one wasp), the difference in phenology between upper and lower nests is less than what we would predict based on the difference in degree-day accumulation (and absent entirely in two of the bees). This suggests that the longer chilling period experienced by insects in lower nests compensated, at least partially, for the delay they experienced in springtime warming. This compensation appears to have been especially incomplete in *C. coerulans*, which showed the greatest difference in phenology between upper and lower nests—supporting the notion that this species is less influenced than others by chilling.

Our results do not prove that chilling affects emergence phenology, although they are consistent with such an effect. Another possible explanation for among-site differences in phenology is that both insects and plants might use a photoperiod cue as an indicator of when heating units begin to “count”. Such a mechanism could also easily explain the lack of nest-height effect seen in *Megachile relativa*, a species that emerges in late summer and could use photoperiod to determine a start date for degree-day accumulation that would fall after even a late snowmelt. Our data cannot rule out either possibility, and in fact the two are not mutually exclusive (and may be employed by different species). We favour the chilling explanation, largely based on previous research on insects (cited above) and the well known vernalization requirement of many plants. The existence of a significant or marginally significant “year” effect in the phenology models of a few of our study species also implicates a factor that, unlike photoperiod, varied between years; however, we cannot rule out a year effect deriving from slight differences among years in our experimental methods or in the sex ratios of emerging bees. Photoperiod also seems an unlikely cue for insects overwintering deep inside wood (though perhaps not impossible; Tauber *et al.* 1986). The relative importance of photoperiod and chilling could be evaluated using a transplant experiment similar to the one we describe here, but with an additional

treatment in which nesting bees are transplanted in spring, after overwintering (and experiencing chilling conditions) at the site of origin. If chilling is important, bees transplanted from low to high sites in spring should emerge later than bees that spent the winter at the high elevation. Conversely, if chilling is unimportant, but photoperiod determines the starting point for heat accumulation, bees at high sites should emerge at the same time regardless of when they were transplanted—provided the spring transplant occurred before the critical daylength.

Implications for phenology modelling

Our dataset is not appropriate for testing the effects of more than a small number of interacting variables, so our results are best viewed as hypotheses awaiting testing under controlled conditions. In reality, factors such as moisture availability, competition, predation, and extreme weather events could affect phenology as well, potentially modulating the effects of cumulative heat units or photoperiod. Even the effect of heat on development rate might be non-linear (see discussion in van Asch & Visser, 2007) or might vary according to the life-stage of the insect (Nealis *et al.*, 1984; Manel & Debouzie, 1997). The most promising way of untangling these effects probably involves an iterative series of field studies and complementary laboratory or growth chamber experiments, the results of each (in)validating and informing the other.

Nevertheless, the data we have so far tentatively suggest a major effect of cool over-wintering temperatures on phenology. Chilling or vernalization effects have been clearly documented in plants (Murray *et al.*, 1989; Henderson *et al.*, 2003) and insects (Kimberling & Miller, 1988; Bosch & Kemp, 2003; Bosch & Kemp, 2004), but this fact seems to be underappreciated by many ecologists monitoring phenology and making projections about climate change impacts (the process-based models such as those reviewed by Chuine [2010] are a notable exception). Simple, single-parameter degree-day models of phenology may fit well for organisms at one location, but these ostensibly “mechanistic” models will have little power to predict phenology under new conditions, when chilling temperatures—not to mention other factors—also vary. Greater awareness of these possible complexities should permit better forecasts of the phenologies of individual species and the possibility of phenological asynchrony between interacting species.

Implications for plant and insect populations

If we are correct in concluding that plants and insects use similar, but not identical, environmental cues to regulate phenology, what are the likely consequences? Our results suggest that, under novel climate conditions, some changes in temporal co-occurrence patterns are possible in our study area. These are more likely to be quantitative effects, with species experiencing reduced or increased overlap with other species, rather than qualitative changes involving complete decoupling of formerly interacting organisms. This is particularly true since our sampling included only plants within 100 m of nest sites; more remote populations with different phenologies might be accessible to insects with longer flight distances. This is especially likely in mountainous habitats with rugged topography. Also, as far as we know, none of the pollinator species in our study is specialized on a single plant species. Hence, these species are unlikely to be strongly affected by shifts in the plant communities with which their phenologies overlap. For example, although *Hoplitis fulgida* missed *Lathyrus* flowering at several sites in 2009, it maintained fairly good synchrony with *Potentilla*, a commonly used pollen source for this bee. However, even in generalist bees, larval growth and survival can vary significantly according to the pollen species in the diet—even among pollen species that adult females are willing to collect for their offspring (Williams, 2003). Studies monitoring the consequences for bee populations of changes in resource use will therefore be an important, but challenging, future step.

The only insect species in our samples that might have been at risk of emerging before *any* plants were available were the early-emerging species of *Osmia*. *Osmia lignaria*, the blue orchard bee, was the earliest species to emerge from our trap-nests in 2008 (mid-May at the low-elevation site; mid-June at the high site in nests transplanted from the low site). Interestingly, emergence of these bees at both sites coincided with the opening of the earliest flowers at each site: The first bees appeared 0 and 5 days after we recorded the first *Mertensia fusiformis* flowers at the low and high sites, respectively. The earliest bee to emerge in 2009 was *Osmia pikei*, a polylectic species (Cripps & Rust, 1985), of which the first individuals appeared 1–2 weeks after the first *M. fusiformis* flowers at each site. Thus, we have no indication that any bee species is emerging “too early” under current conditions, although it remains possible that future climates could produce such a scenario.

From the plant perspective, these observations indicate that flowering before the emergence of any trap-nesting pollinators is a real possibility. This might be especially so in the future, if more rapid snowmelt, followed by temperatures in the 0–5°C range, means that plants get an earlier start to the season without a corresponding advance in insect phenologies. Earlier snowmelt is indeed likely: the proportion of precipitation falling as snow is generally dwindling in the southern Rocky Mountains (Knowles *et al.*, 2006), and, in addition, dust transported from the increasingly arid southwest can decrease surface albedo and increase the melt rate of what snow there is (Painter *et al.*, 2007). However, trap-nesting Hymenoptera are not the only—or even necessarily the most effective—pollinators active early in the season in the Rockies. Ground-nesting bumble bee queens (*Bombus* spp., Apidae) and male *Andrena* spp. (Andrenidae), as well as migratory broad-tailed hummingbirds (*Selasphorus platycercus*) and various species of flower-visiting flies, are often observed before the first *Osmia*. Queen bumble bees are regular visitors to *M. fusiformis*, and all of these taxa frequent other early-flowering species. Earlier flowering of species normally visited by *Osmia* might mean a reduction in the total number of pollinator visits or a change in identity of the main pollinators, but it might not entail a complete loss of pollinator services.

In general, we suspect that organisms inhabiting naturally variable environments such as these subalpine habitats will have evolved strategies for coping with that variability. The parsivoltine, or facultatively semivoltine, life cycle we document here in *Osmia iridis*, *O. tersula*, and *O. tristella* may serve as just such a strategy. The risk-spreading advantage of this life history was suggested by Torchio and Tepedino (1982), who noted that both 1- and 2-year forms could be found in a single *Osmia* nest (this was also the case in our study). At present, we do not know what determines whether an individual spends a second season in diapause, or whether diapause through a third winter can occur as well. In our study, some species failed, or all but failed, to emerge in 2009 (*O. tristella*, *O. tersula*), despite having emerged in numbers from 1-year-old nests in 2008. It is tempting to hypothesize that the cooler, overcast weather of June 2009 might have caused bees to stay in or resume diapause. However, in other parsivoltine *Osmia* species, 2-year bees diapause as larvae in the first winter and do not develop to adulthood until the second summer (Torchio & Tepedino, 1982). In these species, at least, an individual bee's developmental schedule must be determined genetically or maternally, or by environmental cues experienced before the first winter. In any case, the parsivoltine strategy clearly buffers the insect

population against catastrophic reproductive failure in any single year. The major threat to such a strategy would be the occurrence of a succession of bad years. In our study area, warmer and drier years in the last several decades have been associated with periods of midsummer floral scarcity (Aldridge *et al.*, in review), and some plant species in particular have experienced a recent series of bad flowering years caused by spring frost damage to developing flowers (Inouye, 2008). These overall reductions in flowering, which are apparently being exacerbated by climate change, may pose the greater threat to both plants and insects than phenological asynchrony.

Where an organism's survival is closely tied to the phenology of another species, there should be strong selection for the two to use the same cues, or at least cues that have historically been strongly correlated. In the desert bee *Perdita portalis* (Andrenidae), for example, emergence happens in response to increased soil moisture—similar to many desert plants that germinate in response to rainfall (Danforth, 1999). This presumably prevents bees from emerging in years when no flowers bloom, although it does not guarantee that the seasonal timing of flowering and bee activity will exactly coincide. In our system, where most species are generalists, reliance by insects on temperature cues similar to those used by plants may be adequate for ensuring sufficient synchrony with the flowering community as a whole. However, further tests will be necessary not only to corroborate our results but also to determine the extent to which they apply to other members of the plant and insect communities. After that, the next step will be to determine whether the diffuse and imperfect synchrony we have observed is sufficient for maintaining plant and insect populations in the long run.

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Chapter 6

Pollinator experience, neophobia, and the evolution of flowering time

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Abstract

Environmental changes, such as current climate warming, can exert directional selection on reproductive phenology. In plants, evolution of earlier flowering requires that individuals bearing genes for early flowering successfully reproduce; for non-selfing, zoophilous species, this means that early-flowering individuals must be visited by pollinators. In a laboratory experiment with artificial flowers, we presented captive bumble bees (*Bombus impatiens*) with flower arrays representing stages in the phenological progression of a two-species plant community: Bees that had been foraging on flowers of one colour were confronted with increasing numbers of flowers of a second colour. Early-flowering individuals of the second “species” were significantly under-visited, because bees avoided unfamiliar flowers, particularly when these were rare. We incorporated these aspects of bee foraging behaviour (neophobia and positive frequency dependence) in a simulation model of flowering-time evolution for a plant population experiencing selection against late flowering. Unlike simple frequency dependence, a lag in pollinator visitation prevented the plant population from responding to selection and led to declines in population size. Pollinator behaviour thus has the potential to constrain evolutionary adjustments of flowering phenology.

Introduction

Interactions with other species can affect an organism’s ecological and evolutionary responses to environmental change. Theoretical and experimental work has shown that predation and competition can influence demographic responses to changing conditions and limit a species’ ability to adapt to a changing resource optimum (Ives, 1995; Davis *et al.*, 1998; Jiang & Kulczycki, 2004; Johansson, 2008). These results suggest that a failure to consider species

interactions can lead to overly optimistic predictions about the ability of populations to cope with environmental change. However, little attention has been paid to the role of mutualists in enabling or restricting evolutionary responses to changing conditions, although it has been noted that a shortage of mutualists could act as a constraint on the range shifts expected to result from climate change (Possingham, 1993). In this article, we explore how the behaviour of a mutualist can affect a species' evolutionary response to a changing environment.

Timing of reproduction can strongly affect fitness (*e.g.*, Réale *et al.*, 2003; Both *et al.*, 2006; Kudo, 2006). For plants, flowering at a time when soil moisture is adequate can be critical, and, in seasonal environments, fruiting must be completed before the onset of frost or drought (reviewed by Rathcke & Lacey, 1985). Changes in the abiotic environment may lead to shifts in the optimal timing of reproduction and consequent selection on flowering phenology. For example, current climate change is believed to be driving aridification in certain regions (IPCC, 2007; Seager *et al.*, 2007), a trend that may favour plants that complete reproduction earlier in the season (Stinson, 2004; Franks *et al.*, 2007). Flowering time is known to have a genetic basis in several species, and can respond to selection (Mazer & LeBuhn, 1999; Geber & Griffen, 2003; Franks *et al.*, 2007). Of course, for early flowering to evolve, early-flowering individuals must successfully reproduce; for outcrossing, zoophilous species, this requires that early-flowering individuals receive pollinator visits. Thus, predicting a plant's evolutionary response to environmental change requires considering the dynamics of its interactions with mutualists.

Early-flowering individuals within a population are both rare (relative to other plant species flowering in the community at that time) and unfamiliar to pollinators—traits that may cause these individuals to receive relatively few pollinator visits. It is already known that pollinators, like many consumers (Punzalan *et al.*, 2005), tend to forage in a positively frequency-dependent fashion, at least under controlled laboratory conditions (reviewed by Smithson, 2001). Bumble bees (*Bombus terrestris*) faced with arrays of rewarding artificial flowers of two different colours tend to over-visit the more common type, all else being equal (Smithson & Macnair, 1996, 1997). Positive frequency-dependent pollinator visitation could reduce the reproductive success of rare floral morphs of obligately animal-pollinated plants, and therefore has the potential to impose a constraint on floral evolution (Smithson 2001). In nature, rarity is likely to covary with familiarity: rare flower types may also be unfamiliar to pollinators. The avoidance of unfamiliar (often food) objects, known as neophobia, has been documented in birds (Coppinger, 1970) and

many other vertebrates (reviewed by Brigham & Sibly, 1999), but has been largely unstudied in invertebrates. In some cases in which positive frequency-dependent pollinator foraging has been observed in the field (*e.g.*, Hersch & Roy, 2007), the behaviour could result from a combination of preference for the common type (true frequency-dependence) and avoidance of the unfamiliar type (neophobia).

Even if pollinators eventually learn to visit new flower types, a lag in their detection or acceptance of rare and unfamiliar flowers could penalize early-flowering individuals and might impose a constraint on the evolution of early flowering. Such a lag has been inferred from observations in the field that plants often show relatively low pollinator visitation early in their flowering period, and that visitation rates can be more closely correlated with flower densities at an earlier date than with current densities (Thomson, 1981, 1982). However, the existence of this type of hysteresis in plant–pollinator interactions has not been rigorously tested or quantified under controlled conditions.

Here, we test the hypotheses: (i) that bees exhibit a lag in acceptance of novel flowers and (ii) that such a lag could negatively affect a plant population experiencing selection for earlier flowering. To evaluate the first hypothesis, we conducted an experiment with captive bumble bees foraging on artificial flowers of two colours, presented in a sequence that simulated the seasonal progression of flowering in a two-species plant community. We then used computer simulations to evaluate potential effects of the observed pollinator behaviour on the evolutionary trajectory of a plant population.

Methods

Foraging experiments

We conducted two experiments with laboratory-reared bumble bees (*Bombus impatiens* Cresson). In Experiment 6.1, bees had prior experience with both yellow and blue artificial flowers; the results serve as a comparison with the more realistic Experiment 6.2, in which bees were initially naïve to one of the two flower colours.

Study system and experimental design

We acquired bumble bee colonies from Biobest Biological Systems (Leamington, Ontario, Canada). Colonies were connected to a screened indoor flight arena with a gated tunnel that allowed us to control entry and exit of individual bees. Worker bees were given time to learn to forage on 30% (w/w) sucrose solution (henceforth “nectar”) provided in artificial flowers consisting of 1.5 mL Eppendorf tubes with lids removed and artificial “corollas” attached to the mouth of the tube. Bees were individually marked with coloured correction fluid, and those that were consistently willing to forage were used for experiments. We used 6 bees from two colonies in Experiment 6.1, and 12 bees from three other colonies in Experiment 6.2. Pollen and additional nectar were provided directly to the hive as needed.

Experimental flower arrays consisted of 100 blue or yellow artificial flowers embedded in a 162 × 102 cm green foam-core background, positioned such that each flower was 12 cm from its six nearest neighbours. Each flower position was numbered so that an observer could record which flowers had been visited. For experimental runs, flowers were 3 × 3 cm squares of clear polystyrene, spray-painted either blue or yellow (Appendix E), attached to Eppendorf tubes of the same colour. Each bee, over a two-day period, sequentially encountered several arrays (see below) presenting different proportions of blue and yellow flowers; flowers were assigned to positions randomly. These different treatments are referred to as flower-colour “frequencies”. Each bee foraged for four to seven foraging bouts per frequency (the minimum necessary to obtain 100 flower visits after the first foraging bout[s]; see *Data collection and analysis*). Flowers were washed between frequencies. At the beginning of every foraging bout, each flower contained 3 µL of nectar. Flowers were not refilled during foraging bouts, an arrangement meant to mimic dynamics in a small natural patch of flowers, in which flowers can be temporarily drained of nectar.

Experiment 6.1 (bees familiar with both flower colours)

Training: Bees were allowed to forage freely from a training array of two blue and two yellow flowers filled with nectar until reliable foragers were identified. Prior to an experimental run, to ensure approximately equal proficiency on both blue and yellow flowers, the selected bee was allowed to forage for 1–2 bouts on two yellow and two blue flowers, each containing 3 µL of nectar (refilled after being drained).

Testing: Each bee was presented with arrays of five different colour frequencies (10 blue:90 yellow, 20B:80Y, 50B:50Y, 80B:20Y, and 90B:10Y flowers). Arrays were presented in random order to avoid any lag effect that could result from bees experiencing frequencies in increasing order, as they did in Experiment 6.2.

Experiment 6.2 (bees familiar with only one flower colour)

Training: The training array consisted of five flowers with white corollas. Once a reliable forager was identified and accustomed to foraging on flowers containing only 3 μ L of nectar, the white training flowers were replaced by flowers of what was to be that bee's familiar colour (blue or yellow, assigned alternately). These flowers always contained only 3 μ L of nectar at the base of the tube, and bees frequently had to be led into the tube by a trail of nectar drops. We allowed each bee to forage for one complete foraging bout on the coloured training flowers after its first successful (rewarded) entry.

Testing: Each bee was first presented with an array consisting of 100 flowers of the familiar colour. After 4–5 foraging bouts on that array, the bee encountered arrays that comprised a constantly increasing frequency of the novel colour (10%, 20%, 50%, 80%, 90%; ≥ 4 consecutive bouts at each frequency). If bees had still not switched to the novel colour at 90%, they were allowed to forage for one bout on an array of 100% novel flowers.

Data collection and analysis

Because bees often encountered empty (drained) flowers and frequently rejected these flowers without fully entering them, we counted flower visits in two ways: In the first, we counted only visits in which more than half the bee's body entered the Eppendorf tube. In the second, we included all visits in which a bee landed on a flower and faced the tube entrance. In real flowers, these brief inspection visits might not transfer pollen, but they do reflect bees' decisions to investigate. We ran all analyses using both datasets but, because the results are qualitatively identical, we present only the results based on the second (more complete) protocol.

We omitted visitation data from the first foraging bout for each bee at each frequency because we suspected that the first foraging bout would not reflect the “asymptotic” behaviour pattern attained after some initial learning. Bees typically continued to do whatever they had been doing in the preceding foraging bout, and a clear switch to the new flower colour often occurred during

the first bout at a given frequency (Fig. 6.1 shows a representative example). In some cases, the switch did not occur until the second or third foraging bout; in these cases, we only considered data from bouts after the switch. This protocol gives a conservative estimate of any lag in visitation patterns. However, in four cases, bees visited a novel flower during the first foraging bout at a particular flower-colour frequency and visited no others in subsequent bouts at that frequency; omitting the first bout meant ignoring that visit. We believe this is reasonable because a single visit to an outcrossing species would not result in successful pollen transfer.

To estimate the level of frequency dependence when bees had prior experience with both flower colours, we fitted the data from Experiment 6.1 to a curve of the form:

$$Y(A) = \frac{(V \times A)^b}{(V \times A)^b + (1 - A)^b} \quad (6.1)$$

(Smithson & Macnair 1996), where Y is the proportion of visits to yellow flowers and A is the proportion of yellow flowers available (0.1, 0.2, 0.5, 0.8, or 0.9); the curve terminates at (0,0) and (1,1). The parameter b indicates the strength of frequency dependence, with values < 1 indicating negative frequency dependence and values > 1 indicating positive frequency dependence. Values of $V > 1$ indicate a frequency-independent preference for yellow; values < 1 indicate blue preference. The parameters b and V were estimated using the Nonlinear platform in JMP IN v.5.1.2.

To evaluate the strength of the lag effect shown in Experiment 6.2, we treated visitation data as binary (*i.e.*, each visit was to yellow or blue) and conducted a repeated-measures logistic regression on the number of visits made to flowers of a given colour, with “frequency” and “novelty” as continuous and categorical predictors, respectively, and individual bees as subjects. We tested visits to each colour separately (using data from the same six bees from Experiment 6.1 in both analyses). Because these experiments were conducted at different times, the “novelty” treatment is, strictly speaking, pseudo-replicated. Nevertheless, we treat individual bees as the meaningful units of replication, and argue that it is implausible that our results could be due to any conceivable confounding factors. Analyses were conducted using PROC GENMOD in SAS v. 8.02 with a logit link function, and significance was tested using

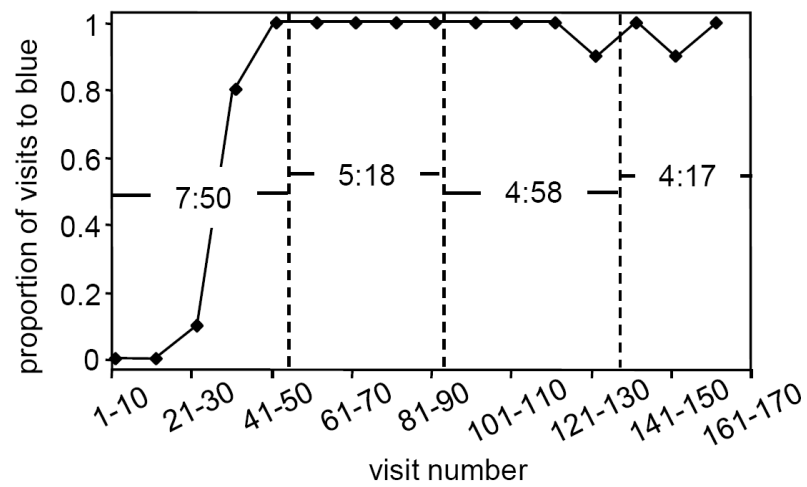


Figure 6.1. Data from a representative bee in Experiment 6.2, foraging on an array of 80 blue (novel) and 20 yellow (familiar) flowers. The visit sequence is broken into 10-visit sections. Dashed lines separate the four foraging bouts, the durations of which (in minutes) are indicated in the figure.

generalized estimating equations (GEEs), which provide a way to deal with correlated measurements in generalized linear models (Liang & Zeger, 1986). When we obtained a significant frequency $\times$ novelty interaction, we tested for differences in the proportion of visits made to novel or familiar flowers at each frequency separately, using a sign test on the number of replicates that lay above the median value for that treatment.

Simulation model

Our model considers the fate of a finite, out-crossing, annual plant population, in which flowering times are genetically determined, experiencing a shift in environmental conditions that alters the “optimal” flowering date. Plants flowering on dates far from the optimum are selected against. Each plant produces one flower that lasts for one time unit (“day”) and can only be pollinated by other plants flowering in that time unit (*i.e.*, there is strict assortative mating by flowering date). Pollinators are generalists; their population is not modelled explicitly and is assumed to be of constant size throughout each simulation run (*i.e.*, their abundance is independent of the abundance of the focal plant). Because the trait of interest is flowering day, we are interested in the total number of plants setting seed on a given day, rather than in the specific identity of these plants; we therefore treat seed set as a cohort-level, not an individual-level, phenomenon.

For any individual plant flowering on day t , the probability of producing seed is determined by the probability that the plant receives a pollinator visit and the probability that the pollinator has visited a conspecific at some time in the past. Visitation probabilities can be modified by frequency dependence and a lag in pollinator response to floral abundance, such that visitation on day t is a sigmoid function of the focal plant’s abundance at time $t - m$. Thus, the probability of pollen transfer for each plant on day t is:

$$P(\text{PT})_t = \beta \left(\frac{(V \times A_{t-m})^b}{(V \times A_{t-m})^b + (N - A_{t-m})^b} \right)^{2-c} \quad (6.2)$$

where β (“pollinator abundance”) is a parameter that modifies the total number of pollinator visits, N is the total number of flowering plants of all species in the community on any given day, A_t is the abundance of the focal plant species on day t , m is the magnitude of the lag in visitation, V and b are parameters describing the strength of preference for the focal species and the strength

of frequency dependence, respectively (as above), and c is a measure of pollinator constancy (Table 6.1). Probability of pollen transfer has a maximum value of 1 (although larger values are mathematically possible for large values of β). Here, constancy is defined simply as the probability that a pollinator is carrying conspecific pollen, and reflects the tendency of pollinators to visit sequences of conspecific flowers, independent of preference or availability (Waser, 1986); we treat this as a separate phenomenon from the lag effect. In the simplest scenario of a single pollinator making two flower visits within the simulated community, c determines the likelihood that this set of flower visits results in successful pollen transfer, given that the first of the two visits is to the focal species. When $c = 1$ (perfect constancy), $P(\text{PT})_t$ is simply the probability of receiving a single visit; when c is 0, visits are independent and $P(\text{PT})_t$ becomes the product of the probabilities of two visits (see also Sargent & Otto 2006 for a similar treatment of constancy). We have not considered details of pollinator visit sequence, number of visits per pollinator, or pollen carryover; however, varying β and c serves the same function, by controlling the total number of successful pollen transfer events at the level of the whole cohort, given the visitation probability determined by frequency-dependence and the time lag.

For each pollen transfer event occurring on a given day, the number of surviving seeds produced by the recipient plant is described by:

$$S_t = \exp \left[p \left(1 - \frac{A_t}{K} \right) \right] \times \exp \left[\frac{-(t - t_o)^2}{2s^2} \right] \quad (6.3)$$

where the first part of the expression describes density dependence, with e^p being the number of seeds produced when the number of plants flowering on day t is far from the carrying capacity, K , for each day (as in the Ricker map; Mangel 2006). The use of a daily K rather than a carrying capacity for the whole population is necessary to prevent population oscillations; note that within-day density dependence tends to increase seed set for plants at the tails of the flowering distribution. This might be reasonable if, for instance, competition for the resources needed for seed maturation were most intense for plants flowering during the population peak. The second part of Equation (6.3) determines the proportion of seeds surviving as a Gaussian function with mean t_o and variance s^2 , such that survival decreases as the flowering date of the parent plant

Table 6.1. Model parameters and variables.

Symbol	Range of values	Description
A_t	≥ 0	Abundance of focal species at time t
b	> 0	Frequency-dependence exponent $b < 1$: negative frequency-dependence $b > 1$: positive frequency-dependence
β	≥ 0	Pollinator abundance
c	$0 \leq c \leq 1$	Pollinator constancy: probability a pollinator carries conspecific pollen
h^2	$0 \leq h^2 \leq 1$	Heritability of flowering time
K	100	Maximum number of individuals of focal species flowering on any day (carrying capacity)
m	≥ 0	Time lag
N	120	Total number of flowering individuals of all species on any day
p	> 0	Determines the number of seeds produced per successful pollen transfer before selection
s^2	> 0	Variance of normal distribution defining abiotic selection
σ	> 0	Standard deviation in offspring phenotype
t	$1 \leq t \leq 30$	Flowering day
t_0	15	“Optimal” flowering day favoured by abiotic selection
V	≥ 0	Pollinator preference $V < 1$: preference for other species $V > 1$: preference for focal species

becomes more distant from the optimal date t_o . Survival is modelled as a deterministic process, while pollen transfer is probabilistic.

The phenotype (flowering date) of the surviving offspring of all plants that flowered on day t is determined by the trait's heritability, h^2 , which dictates mean offspring phenotype, according to:

$$\text{mean offspring phenotype} = (1 - h^2) \times (\text{mean phenotype of parental generation}) + h^2 \times (\text{mid-parent phenotype}) \quad (6.4)$$

(Roff, 1997). Paternal and maternal trait values are identical in this model, because of the simplifying assumption of completely assortative mating. Variation around the mean is controlled by a second parameter, σ . Thus, each individual offspring produced for plants flowering on a given date is assigned a flowering date probabilistically, according to a normal distribution around the mean offspring phenotype with standard deviation σ .

For simulation runs, the plant population started with a normal distribution of flowering dates with mean = day 20, s.d. = $\sqrt{10}$, and carrying capacity = $K = 100$ plants; N , the total number of flowering plants in the community on any day, was set at 120. We selected starting parameter values such that the population remained stable in the absence of directional selection. We then set the optimal flowering date $t_o = \text{day 15}$, and ran the algorithm for 10 generations, by which point the population's size and flowering distribution had stabilized. We systematically varied the pollinator visitation parameters β (abundance), b (frequency-dependence), m (time lag), V (preference), and c (constancy), as well as the inheritance parameters h^2 and σ . The response variables of interest were final population size and peak flowering date of the population. For each parameter combination, we ran the model 100 times to generate confidence intervals. We also ran simulations using different model assumptions; in particular, we replaced the Gaussian abiotic selection function by a step function to simulate truncation selection, and we modified the density-dependence function so that density-dependence operated over the whole population, rather than within flowering days. Because these modifications did not alter any of our conclusions, the results we report are for the original model. The simulations were implemented in MATHEMATICA v. 5.0.

Results

Foraging experiments

Experiment 6.1 (bees familiar with both flower colours)

The proportion of visits to yellow flowers as a function of their relative abundance in the array is shown in Fig. 6.2a (open symbols). The fitted value of b is 1.23 ± 0.09 (mean $\pm$ s.e.), indicating positive frequency dependence. The fitted value of V is 0.89 ± 0.07 , indicating a weak (non-significant) preference for blue.

Experiment 6.2 (bees familiar with only one flower colour)

Flowers of a given colour received a lower proportion of visits when that colour was unfamiliar compared with when bees had prior experience of both colours (Table 6.2, Fig. 6.2). The cost of novelty was most apparent when a colour was also rare (frequency $\times$ novelty interactions, $P < 0.05$; Table 6.2): sign tests detected significantly lower visitation to novel than familiar yellow flowers only when these made up 50% or less of the array, and to novel blue flowers when these made up less than 20% of the array (sign tests, $P = 0.031$). This difference between yellow and blue was a result of greater variation among individual yellow-trained bees in when they switched to the novel colour. When bees had been trained on blue, yellow flowers received no visits until they made up 80% or more of the array, whereas certain bees visited unfamiliar blue flowers when these were only 20–50% of the array. Overall, bees were more reluctant to visit novel yellow than novel blue flowers: the mean frequency at which $\geq 50\%$ of visits were to the novel colour was 93% for yellow and 72% for blue (Kruskal–Wallis test, $\chi^2 = 5.77$, $df = 1$, $P = 0.016$; Fig. 6.2).

There was striking behavioural variation among individual bees (Appendix F): Before switching to the unfamiliar flowers, some bees would repeatedly revisit drained flowers of their preferred colour, making as many as 38 consecutive unrewarded visits, and occasionally leaving the array to fly around the perimeter of the cage. One bee refused to visit unfamiliar yellow flowers until they made up 100% of the array, and two additional bees still refused yellow flowers when given no choice (these returned to the colony and abandoned foraging). Other bees visited unfamiliar flowers without having encountered a series of drained, familiar flowers. Once bees had begun to visit the novel flower type, they quickly learned that these flowers were rewarding; some bees

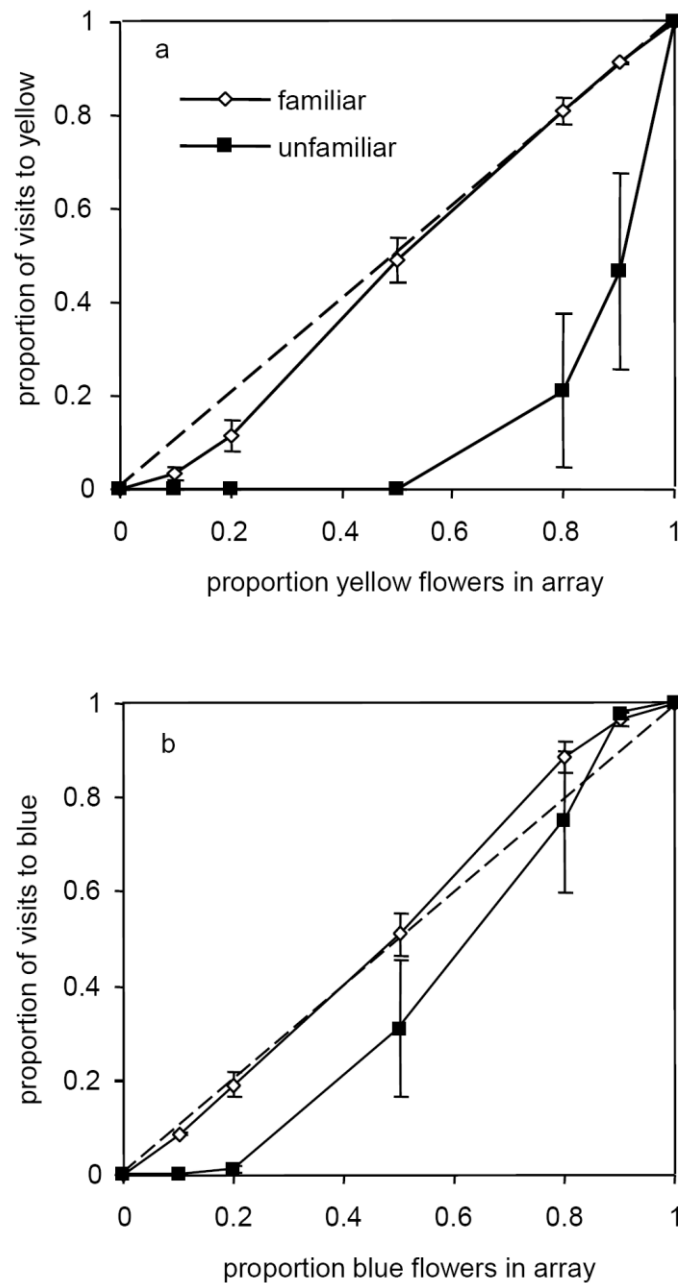


Figure 6.2. Proportion of visits received by (a) yellow and (b), blue flowers, according to the frequency with which that colour occurred within the array. Open diamonds represent means for bees with prior experience of both flower colours, and filled squares represent means for bees that were unfamiliar with that colour ($N = 6$ bees for each treatment, but note that the “familiar” data points in both plots represent the same six bees). The dashed line is the 1:1 line. Error bars are ± 1 s.e.

Table 6.2. Results of repeated-measures logistic regressions testing effects of flower-colour frequency and novelty on number of bee visits to each colour (frequency treated as continuous).

Explanatory variable	Z	df	<i>P</i>
Visits to yellow:			
Frequency	3.13	1	0.002
Novelty	2.56	1	0.011
Novelty × frequency	2.23	1	0.026
Visits to blue:			
Frequency	6.89	1	< 0.0001
Novelty	2.97	1	0.003
Novelty × frequency	2.38	1	0.017

even developed a preference for the novel type, which was more reliably rewarding than the depleted, familiar type (Appendix F).

Simulation model

For a population under selection for earlier flowering, increasing the frequency-dependence parameter, b , slightly reduces the population response to selection and causes a decrease in population size (Fig. 6.3a). This effect is small at the level of frequency dependence observed in our experiment ($b \cong 1.2$), but is more apparent for $b = 1.4$ – 1.8 , values obtained by Smithson and Macnair (1996; 1997).

Introducing a one-day lag in pollinator visitation causes a dramatic decline in the population response to selection and leads to reductions in population size and frequent extinction (Figs. 6.3b, 6.4). This is true even without frequency dependence ($b = 1.0$), although the reduction in population size is less severe when rare plants are not under-visited. A partial lag, simulated using the mean of visitation probabilities obtained with ($m = 1$) and without a lag ($m = 0$), produces an intermediate reduction in population abundance (Fig. 6.3b).

These qualitative conclusions are unchanged by varying the inheritance parameters h^2 or σ ; populations are consistently significantly reduced by inclusion of a one-day lag because of a failure to respond to selection. For almost all parameter combinations, the effect of a one-day lag is substantially greater than the effect of a low level of frequency-dependence ($b = 1.2$), which causes no detectable decline in the response to selection. Truncation selection produces identical results. Predictably, increasing pollinator abundance (β), constancy (c), or preference for the focal species (V), or introducing autogamous seed production (seed set independent of pollinator visitation), increases seed set for all plants and consequently increases plant population size, but does not affect the population's ability to evolve earlier flowering.

Discussion

When our *Bombus impatiens* workers had prior experience with both flower colours, they showed positive frequency-dependent foraging behaviour, preferentially visiting the more common flower colour. These results qualitatively match those previously obtained with *B. terrestris* (Smithson & Macnair 1996). However, when we incorporated a more realistic

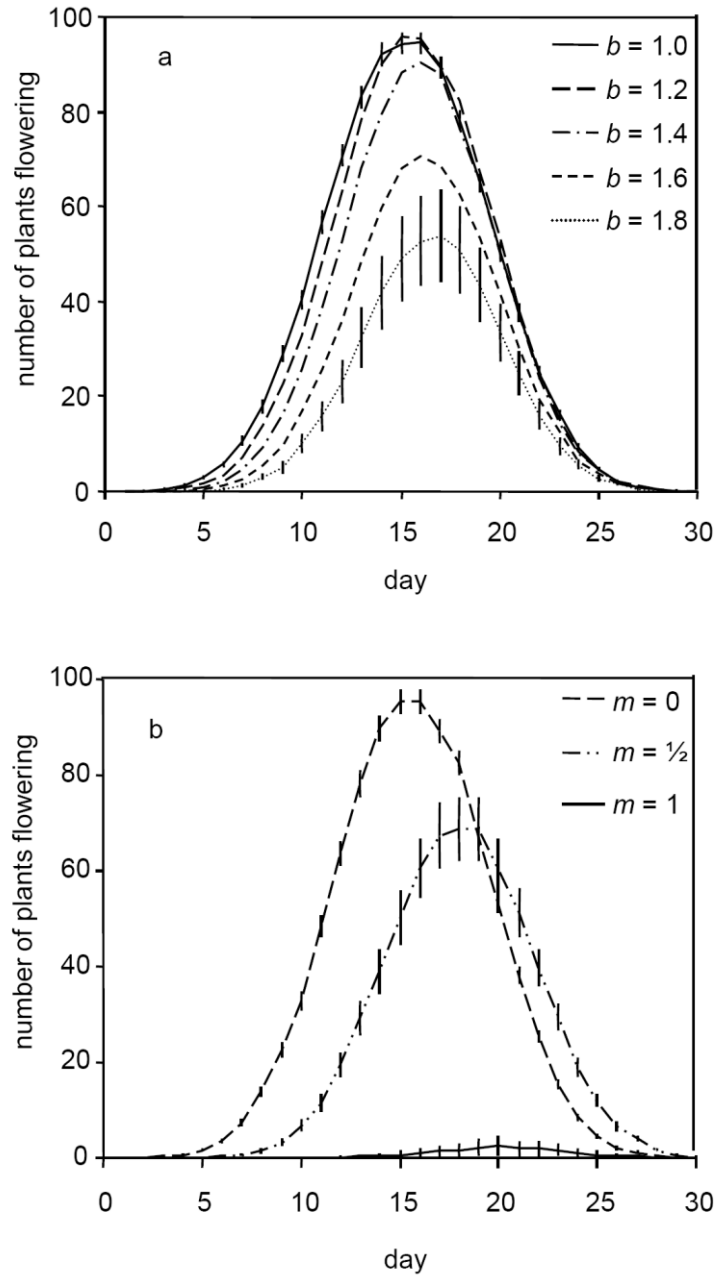


Figure 6.3. Effects of varying (a) frequency-dependence, b , and (b) the lag parameter, m , on the population flowering distribution after 10 generations of selection. The starting mean flowering day is 20; the new “optimal” flowering day, t_0 , is 15. Pollinator visitation probabilities for $m = 1/2$ were obtained by averaging those for $m = 1$ and $m = 0$. Parameter values are: $s^2 = 30$, $h^2 = 0.7$, $\sigma = 3$, $p = 5$, $c = 0$, $\beta = 1$, $V = 1$. For (a), $m = 0$; for (b) $b = 1.2$. Error bars are 2 s.e., and are shown in (a) only for $b = 1.0$ and $b = 1.8$ for clarity.

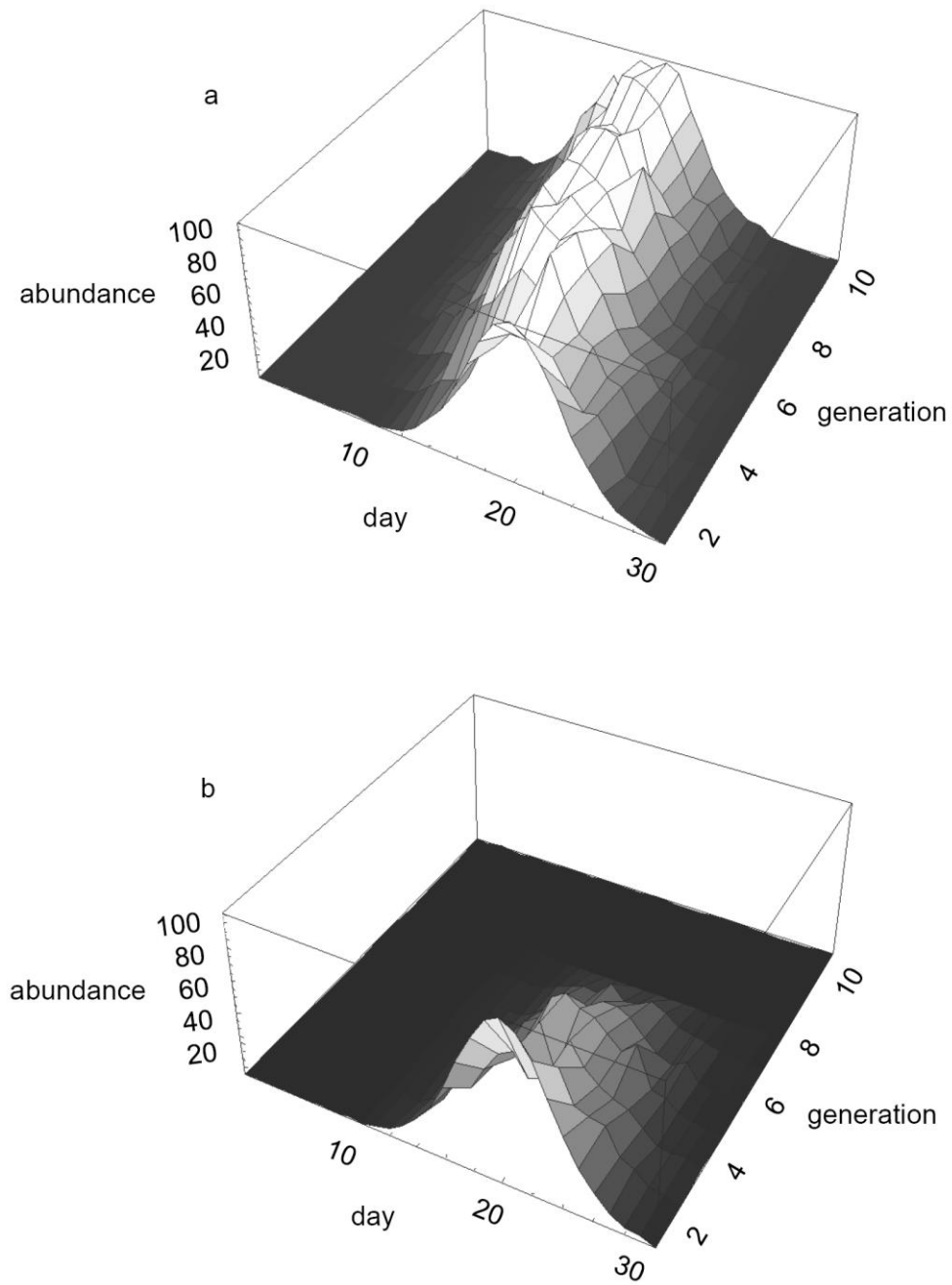


Figure 6.4. Examples of evolutionary trajectories for the population flowering curve over 10 generations of selection for earlier flowering at different levels of the lag parameter, m . (a) $m = 0$, (b) $m = 1$. Other parameters as in Fig. 6.3, and $b = 1.2$.

sequence of foraging environments that simulated temporal change in a simple flowering plant community, we observed a marked difference in behaviour: Bees were less likely to visit a flower type that they had not previously learned to associate with a reward, despite the similarity of the flower types in every respect except colour, and despite the repeated discouragement of encountering familiar flowers that had been emptied of nectar. A similar lag in use of a food source has been observed in a laboratory study with wild-caught bumble bees (*B. ternarius* and *B. terricola*). Bees that had been trained to recognize one colour of artificial flower as rewarding and another as unrewarding were slow to switch allegiances when the identity of the rewarding type was reversed (Heinrich *et al.*, 1977). For an animal foraging in a changing and unpredictable environment, there should be an “optimal forgetting rule” that allows it to adjust its foraging decisions on the basis of more recently acquired—but imperfect—information (Mangel, 1990). The forgetting rate of our bees was apparently too slow to allow precise tracking of changes in resource availability.

It is unclear to what extent these results represent pollinator behaviour in a more natural setting. However, there is some evidence of conservatism in the foraging behaviour of wild bees: Heinrich (1976) conducted flower removal and addition experiments in the field and observed strong fidelity of worker bumble bees to the species on which they had originally been foraging, as well as apparent avoidance of unfamiliar species. Kawaguchi *et al.* (2007) also found that bumble bees (*B. diversus*) rejected flower bouquets of an unfamiliar species more often than bouquets of familiar flowers. In our experiment, when bees encountered a series of unrewarding flowers, they often left the array and flew around the perimeter of the flight cage before visiting a novel flower. This behaviour suggests that, given a larger foraging area, some bees would be more likely to search for a new patch of familiar flowers than to visit a new species when their preferred flowers become locally depleted. This suggestion is supported by Heinrich’s (1979b) observation that bumble bees confined to an outdoor flight cage eventually sampled flowers of species newly introduced to the cage, but that bees released from the cage tended to maintain their original flower preferences by foraging more widely. Although our artificial flowers were undoubtedly less attractive than real ones, their morphology was simple; complex flower types with concealed nectar or pollen rewards might be more likely to experience a lag in visitation because of the time needed for bees to learn new flower-handling techniques (Lavery, 1980).

Clearly, for bumble bee colonies to survive, they must be capable of using a series of different plant species throughout a growing season. However, the colony may track changing resource availability even if behaviour of individual bees shows a lag, as long as newly emerging workers take advantage of new food sources. Our results suggest, though, that as long as a familiar species remains in bloom, new species will likely be under-visited by experienced workers. Furthermore, the available literature suggests that bumble bees and other social bees often copy the flower choices made by other bees, particularly when faced with unfamiliar flowers (Slaa *et al.*, 2003; Leadbeater & Chittka, 2005; Worden & Papaj, 2005; Kawaguchi *et al.*, 2006, 2007).

Insects other than worker bumble bees may be less reluctant to explore and exploit new floral resources. The foraging behaviour of non-apid pollinators is relatively unstudied, and we have little information about lags in resource use by flies or solitary bees (though see Thomson 1981). However, butterflies will preferentially visit flower species with which they have prior experience (Lewis, 1986), and many solitary bee species are pollen specialists, though they may visit multiple species for nectar (Michener, 2000). Flower constancy has also been noted for several taxa (Goulson, 2000)—though constancy and the neophobia we have documented here are separate phenomena. Honey bees display marked flower-colour constancy, and, given a choice, tend not to sample new colours of artificial flowers even when the new flowers are more rewarding (Hill *et al.*, 1997). By comparison, bumble bees are behaviourally flexible and intelligent foragers (Chittka *et al.*, 2001; Gegear & Laverty, 2004), making our results somewhat surprising. In fact, bumble bees have been considered models of optimal foraging behaviour, obeying seemingly rational decision rules that maximize the rate of individual energy intake (*e.g.*, Pyke, 1978; Zimmerman, 1981). Our results suggest that behaviour of individual workers can deviate substantially—if temporarily—from “optimality”, and models that fail to consider lasting effects of prior experience are over-simplified. Further experiments will be necessary to document the severity and generality of pollinator neophobia. Flight cage studies in which additional floral traits (*e.g.*, nectar volumes, odour) are manipulated would help determine factors that could increase or decrease a bee’s willingness to sample new flowers. Field studies documenting visitation by wild pollinators to arrays of familiar and novel (*e.g.*, non-native) flowers would be particularly informative.

Our simulations show that pollinator avoidance of early-flowering plants within a population can impose a significant constraint on evolution of earlier flowering phenology. In the context of the

evolution of aposematism and Batesian mimicry, neophobia and learning are recognized to be important (Coppinger, 1969; Servedio, 2000; Puurtinen & Kaitala, 2006), because predator avoidance of unfamiliar food items facilitates the spread of conspicuous warning colouration in prey populations. The possibility that an analogous phenomenon (but with opposite evolutionary consequences) might play a role in floral evolution appears not to have attracted previous theoretical or empirical investigation—perhaps in part because existing interspecific variation shows that evolutionary adjustments in flowering time have occurred in the past. Our results underscore the need to consider interactions with mutualists when attempting to predict adaptive responses to environmental change.

Our conclusion that pollinator lags in conjunction with selection against late flowering can have negative impacts on plant populations is robust to most changes in model parameters, although numerous factors (*e.g.*, pollinator abundance and constancy) can mitigate the effect on population size. In particular, if the newly flowering species were more rewarding to pollinators than the species already in bloom, the additional pollinator visits received during the rest of its flowering season could make up for those lost by the earliest individuals. Alternatively, if the second species resembled the first, the magnitude of the lag might be lessened (*cf.* Gumbert, 2000). Because colour generalization does not preclude constancy (Chittka & Wells, 2004), the benefit of additional pollinator visits would not necessarily be negated by the cost of interspecific pollen transfer (but see Kunin, 1993).

Our simulations assume a brief flowering duration, so that even a pollinator lag that is short relative to the population flowering period has dramatic consequences for the earliest individual plants. In reality, even if flower longevity is often brief (Primack, 1985; Stratton, 1989), lifetime reproductive opportunities of the whole plant are unlikely to be restricted to a single short-lived flower. In addition, the lag effect we have simulated is time-dependent rather than experience-dependent: that is, the lag is no less severe if the plant population has a positively skewed flowering distribution. Such a distribution—or a greater investment in floral rewards early in a plant's flowering period—might tend to attract pollinators to the new flowers and reduce the delay in resource tracking (Thomson, 1985; Kato & Sakai, 2008). Nevertheless, our simulation results demonstrate the possibility for a severe reduction in a population's capacity for adaptation—and a consequent decline in population viability—with even a partial reduction in the probability of visitation for the earliest-flowering individuals. This suggests that plants with

brief flowering duration, growing in small populations, and experiencing rapid environmental change, may be susceptible to pollen limitation of the evolution of flowering time.

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Chapter 7

Concluding discussion

In this thesis, I have explored various ways in which plant and pollinator phenology interact to influence the pollination process, and ways in which climate change could alter that interaction. One of the motivations for this research was the frequently voiced concern that synchrony between plants and pollinators, in high-elevation communities in particular, could be disrupted by climate change. Here I evaluate this scenario in light of my results and touch on some other mechanisms, highlighted in this thesis, by which climate change could influence pollination. I conclude with some thoughts on future research directions.

Species interactions under climate change

Climate change can be expected to produce asynchronies between interacting species if those species respond differently to environmental change. The word “cue” has often been used in this context, by myself and others (*e.g.*, Visser *et al.*, 1998; Winder & Schindler, 2004; Hegland *et al.*, 2009): interacting species that use different cues to regulate phenology are at risk if those cues are not changing in parallel. The word implies a single discrete event that releases an organism from dormancy, initiates growth, or triggers a behaviour such as leaving the nest to forage. Although some life-history events in some species might indeed be triggered in this way, a more complex and cumulative process is probably more common (see Tauber *et al.*, 1986; Körner & Basler, 2010 for examples). A cue for flowering might be, for example, a particular number of degree-days under a particular photoperiod. It is important to recognize that we usually have only correlational evidence for an association between a particular “cue” and the life-history event it supposedly regulates, rather than a complete understanding of the underlying physiological mechanism. If the climate is reasonably stable, establishing a correlation is sufficient for practical matters such as scheduling agricultural procedures or field research. But if climate change causes historically correlated climatic variables to become dissociated, distinguishing between mechanisms (“true cues”) and mere correlates is important.

A recurring theme in this thesis has been the possibility of diverging trends in subalpine snowpack and spring temperatures, and the potentially greater sensitivity of plants than pollinators to timing of snowmelt. In work conducted at the Rocky Mountain Biological Laboratory, date of snowmelt is often used as a predictor variable for quantifying effects of climate change on plant communities (*e.g.*, Harte & Shaw, 1995; Price & Waser, 1998; Inouye *et al.*, 2000; Inouye, 2008; Lambert *et al.*, 2010; see also Chapter 2). This is in part because local long-term snowpack data are available and local temperature records are not; but it is also because snowmelt date works well (*e.g.*, Dunne *et al.*, 2003). Flowering phenology of many plants is certainly correlated with timing of snowmelt, but is it really a “cue” for flowering? Melting snow releases water, but this is a gradual process; and disappearance of snow does not induce plant growth unless accompanied by temperatures above freezing (Billings, 1987). Conversely, some plants in these habitats can flower beneath the surface of melting snow, where temperatures can hover just above 0°C (Kimball & Salisbury, 1974; Richardson & Salisbury, 1977). Regression analyses that have found a stronger influence of snowmelt than temperature on flowering time (Dunne *et al.*, 2003; Høye *et al.*, 2007) have been based on local snowmelt data but have used air temperature measurements from a more distant weather station. Furthermore, the temperatures may be averaged over an arbitrarily selected time period. These procedures are unlikely to represent the most relevant temperatures for the plant, and the importance of snowmelt may therefore be overestimated. The phenology modelling that I have done is nothing more than another correlative approach; it does not rule out a cueing function of snowmelt. However, the results of Chapter 5 demonstrate that the type of degree-day model that works for predicting insect phenology does at least as well for several co-occurring plants. Temperature, therefore, is no less a “cue” for plant phenology than it is for insects.

That said, the precise thermal requirements for development to emergence or flowering are very likely to differ—not only between plants and pollinators, but also, to a lesser extent, within each of these groups (*cf.* Fitter *et al.*, 1995; Bradley *et al.*, 1999; Miller-Rushing *et al.*, 2007). These differences among species (*e.g.*, in threshold temperatures for development, or in sensitivity to chilling or vernalization) are probably responsible for some of the differences in co-flowering patterns between early- and late-snowmelt years described in Chapter 2, although species-specific effects of snowpack on total flower production or other aspects of the flowering schedule must also have contributed. Generally higher threshold temperatures for insects than

plants may also cause plant phenology to advance beyond that of insects when an early thaw is followed by low—but not sub-zero—temperatures. This type of outcome was suggested by the trend towards early-season pollen-limitation in *Mertensia fusiformis* in 2007, an early-snowmelt year (Chapter 3), and by the lag in *Hoplitis fulgida* emergence relative to flowering of *Lathyrus leucanthus* in 2009 (Chapter 5). These asynchronies—if they warrant that name—are not dramatic. However, a more troubling case of decoupling between subalpine plants and pollinators has been documented in a co-occurring species, the early-flowering *Erythronium grandiflorum*, which seems increasingly to be flowering too early for its pollinators (Thomson 2010). Together, these results provide support for a scenario in which changes in pollinator emergence lag behind the advancing phenology of spring-flowering plants, in some cases reducing plant fitness. This is in contrast to the prospect, raised by Inouye *et al.* (2000), of insect emergence before flowering. It is still theoretically possible that an unusual combination of heavy spring snowpack and unseasonably high temperatures could induce some pollinators to emerge from above-ground nests before flowering, but the anticipated reductions in mountain snowpack make such a situation unlikely.

In addition to differing somewhat in temperature sensitivity and in the microenvironments they inhabit, plants and pollinators also, more obviously, differ in motility. For this reason, small-scale patchiness in snowmelt affects flowering phenology of *Mertensia fusiformis* plants—perhaps indirectly, through its effects on temperatures at the soil level—without affecting phenology of the bees that visit them (Chapter 4). This patchiness was irrelevant to the phenology models developed in Chapter 5 because that study integrated flowering phenology over a broader scale and a larger population. However, the smaller-scale variation is relevant in influencing which flower visitors will be active during the flowering periods of particular plants.

An interesting question, then, is whether particular sub-populations of *M. fusiformis*, which may be attended by different suites of pollinator species, maintain synchrony across years with that same set of pollinators. Consistency in pollinator species identity (or at least in pollinator proboscis length) would be a precondition for the adaptation in floral morphology that I postulated—but failed to detect—in Chapter 4. Possibly, in years when snow melts especially early but temperatures remain low, plants in relatively early-melting sites might receive few pollinator visits, and even those in late sites might flower before female *Osmia* emerge. A few

more years of observation would be necessary to evaluate this idea and to study its consequences for selection on floral morphology.

Consequences of mismatch

My results suggest that fluctuations in phenological overlap within subalpine meadow communities do occur under the current range of climate variation, and that these could become more pronounced as the climate changes further. Clearly, we would like to know the consequences of such changes for plant and pollinator populations. Although my thesis was not primarily aimed at detecting demographic effects of climate change, I will offer a few ideas.

In subalpine communities, both plants and bees are, for the most part, generalists, so that changes in phenology that disrupt synchrony with one species of interaction partner will probably not be catastrophic. (Some plants may be pollinated exclusively by bumble bees, and some solitary bees take pollen only from composites, but I know of no further examples of specialization in this habitat. However, host associations are not known for all bee species.) The most likely exceptions to this robustness are species that flower or emerge at the very beginning or end of the growing season and risk losing all interaction partners—as illustrated by *Erythronium grandiflorum*. Changes in the relative abundances of a plant's flower visitors or its co-flowering competitors might cause reductions in seed production or offspring quality, and perhaps consequent selection on floral traits or phenology. But such quantitative changes seem unlikely to produce the near-total reproductive failure that can be caused, for example, by an unseasonal frost (Inouye 2008). It is even possible that phenological shifts could reduce the opportunity for interaction with an ineffective mutualist (*i.e.*, one that is relatively parasitic) in favour of a more effective one (*cf.* Thomson & Thomson, 1992). Similarly, change in the floral community to which bees have access seems unlikely to cause immediate starvation, as adult bees require only nectar for survival. It might force female bees onto less preferred pollen sources, which could reduce the size and survival of their offspring (Williams, 2003). However, in some cases, bee larvae grow *better* with novel pollen added to their diet (Williams 2003); so, giving adult bees no choice but to forage on unfamiliar flowers could, sometimes, have unexpectedly beneficial outcomes. Regardless of whether the effects of phenological change are positive or negative, they are likely to be neither immediate nor easy to detect.

My studies of *Mertensia fusiformis* suggest that this species, at least, is well equipped to withstand temporary pollinator shortages, sharing with many other taxa an ability to adjust floral longevity in response to pollination. In fact, adaptive plasticity in flowering schedules could have implications beyond those discussed in Chapter 3: If plants were able to respond to pollinator scarcity by delaying flower opening, extending the lifetimes of opened flowers, or extending new flower production later into the season, this would tend to mitigate any initial asynchronies, both for the insects and the plants. This is not far-fetched: In female *Silene latifolia*, for example, schedules of flower initiation are influenced by pollination of earlier flowers (Meagher & Delph, 2001). Having many flowers, and being flexible in when and for how long they are deployed, would also buffer plants against pollen shortages due to pollinator avoidance of novel food sources (*cf.* Chapter 6); however, it would not necessarily facilitate the adaptive adjustment of flowering time that is threatened by that behaviour. The types of plasticity that I documented in *M. fusiformis* are appropriate for plants that have evolved in such a naturally variable environment.

Effects of plant–pollinator asynchronies may be more pronounced in communities with a greater proportion of specialists. One might expect specialists to have evolved to use the same emergence cues as their partners, so that synchrony would be unaffected by climate change. One case study suggests otherwise: Mayer & Kuhlmann (2004) noted emergence of specialist, oil-collecting bees (*Rediviva* spp., Melittidae) in a drought year in South Africa when their flowers were unavailable; these bees were presumably doomed to reproductive failure. This was a surprising finding, given the commonness of drought in this region. It is possible that the bees have evolved partial cohort emergence (like the parsivoltinism I documented in Chapter 5) as an alternative, bet-hedging strategy to prevent entire generations having zero fitness due to asynchrony (*cf.* Danforth, 1999). More generally, though, occasional asynchronies may be an unavoidable consequence of interacting species frequently differing in thermoregulatory abilities and microhabitat use. If this is the case, isolated incidents of mismatch are not necessarily harbingers of climate change (Singer & Parmesan, 2010). However, monitoring relatively specialized interactions to detect changes in the frequency of mismatches would be worthwhile, since the demographic effects of mistiming could be severe.

Constraints on adaptation to changing climate

In Chapter 3, I investigated selection on flowering time in *M. fusiformis*. Early snowmelt and flowering in 2007 was associated with low seed set in the earliest flowers. This was likely due to direct negative effects of cold temperatures on floral tissue (which, as shown in Chapter 4, can reduce seed set even of flowers that appear undamaged), perhaps exacerbated by pollen limitation. These penalties of early flowering counteracted indirect selection for early flowering driven by the correlation between size and flowering onset. This result comes from only a single population in single year, but if it is an indication of future trends, it suggests that delayed flowering could increasingly be favoured in this plant. The likelihood of that outcome depends on how other components of the environment, such as the phenology of potential competitors and the severity of summer drought, also change.

I did not examine selection on emergence phenology of bees, although I believe this will be an important area for future research, especially in areas where asynchrony with floral host-plants seems to be an important threat. In principle, the short generation times of insects should permit relatively rapid evolutionary adjustment to changing conditions. However, factors other than synchrony with a food supply can influence selection on life history, making predictions of evolutionary trajectories more complex. For example, rates of attack by *Stelis montana* (a brood parasite) on *Osmia lignaria* nests are reported to increase over the season (Torchio, 1989), potentially favouring individuals that nest earlier (and explaining the early flight season of this species). Sexual selection could also influence the evolution of bee phenology: Male insects that emerge early typically have access to more and higher-quality mates (Wiklund & Fagerström, 1977; Kleckner *et al.*, 1995); if this favours early emergence in males but not in females, sexual conflict over phenology could result (*cf.* Møller *et al.*, 2009).

In Chapter 6, I presented a largely theoretical argument for another factor that could complicate simplistic forecasts of adaptive evolutionary responses to climate change. The conclusion that pollinator avoidance of rare, novel flower types can counteract selection for earlier flowering still awaits testing in the field. Also, this purely behavioural source of “mismatch” between early-flowering plants and pollinators needs to be integrated with the numerical mismatch dealt with elsewhere in this thesis: The model in Chapter 6 assumes constant pollinator abundance

throughout the flowering season; the actual phenology of pollinator activity could either mitigate or amplify the effects predicted by the model.

Conclusion

I have attempted to add some empirical data, and a theoretical analysis, to the large number of verbal arguments about climate change effects on plant–pollinator interactions. The empirical work described here provides some reassurance that the plants and pollinators I have studied are not in grave danger from phenological responses to climate change. This is, of course, a tentative conclusion; a 2- or 3-year study cannot hope to encompass the full range of historical climate variability, let alone that which can be expected in the future. Still, I suspect that the more direct effects of changing climate—hotter temperatures, more severe drought, and, perhaps, increasing risk of frost exposure—will have more important consequences for the viability of plant and pollinator populations than will shifts in the phenologies of mutualists and competitors. However, these phenological changes are likely to interact with changes in abiotic threats to affect populations in ways that have only been hinted at in this thesis.

Of the unanswered (or only partially answered) questions arising from my work, those that seem most pressing are the following: First, what is the mechanistic basis of emergence phenology, not only for solitary trap-nesting bees but for other pollinators as well? My investigations have produced what are, to my knowledge, the first phenology models for bees. (Their absence from the literature so far is surprising, given the economic and ecological importance of these insects.) However, as I have argued in Chapter 5, these models need further validation. A worrisome thought that accompanies all such model-selection analyses is that one is always limited by one's imagination (and the prior literature) in the set of models one analyzes. Experiments that test the effects of particular variables will be helpful, but the number of possible weather variables that could be tested is infinite. One useful, complementary approach would be to subject nesting insects to temperature regimes that simulate predicted future climates. If emergence is well predicted by an existing phenology model, this provides additional support for that model. Along the same lines, there seems to be much to learn about the basic biology and prevalence of partial cohort emergence, or parsivoltinism, in bees. Bet-hedging strategies like this one could be critically important in the context of increasingly variable climates (*cf.* Childs *et al.*, 2010), but they are by their unpredictable nature especially challenging to study.

A second major area for future research, as I have suggested above, concerns the fitness and demographic consequences of phenological changes. For bees in particular, because we still have an incomplete understanding of their foraging ranges, natal dispersal distances, and abilities to accept and use unfamiliar food sources, it is difficult to know what would constitute biologically significant asynchrony in a realistic, heterogeneous environment. It would also be useful to know what sorts of evolutionary responses to such asynchrony are possible. Research along these lines is a logical next step.

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Appendix A

Mean peak flowering dates (averaged over plots and years) and regression slopes for the 15 most common species with flowering periods overlapping that of *Lathyrus leucanthus*. Slopes of linear regressions between snowmelt date and peak flowering date, flowering duration, or flowering intensity are given (as number of days change in flowering period, or change in number of flowers/inflorescences, per day of delay in snowmelt). Significant relationships ($P < 0.05$) are indicated by asterisks (*). Flowering intensity was 4th-root transformed for significance testing, but slopes are from untransformed data.

Species	Peak flowering date	Slope of regression on snowmelt date		
		Peak	Duration	Intensity
<i>Mertensia fusiformis</i> Greene	7 June	0.68*	0.04	3.4*
<i>Mahonia repens</i> (Lindl.) G. Don	8 June	0.72*	-0.14*	-2.5
<i>Androsace septentrionalis</i> L.	16 June	0.74*	0.09	-0.4
<i>Delphinium nuttallianum</i> Pritz.	19 June	0.56*	0.09	7.8*
<i>Amelanchier alnifolia</i> Nutt.	20 June	0.58*	-0.02	1.9
<i>Arabis drummondii</i> Gray	23 June	0.64*	-0.23	-0.5*
<i>Draba aurea</i> Vahl	27 June	0.58*	-0.19	-0.4
<i>Lathyrus lanszwertii</i> Kellogg var. <i>leucanthus</i> (Rydb.) Dorn	28 June	0.69*	0.09	2.7*
<i>Lupinus polyphyllus</i> Lind. var. <i>prunophilus</i> (Jones) Phillips	6 July	0.43*	0.08	6.2*
<i>Erysimum asperum</i> (Nutt.) DC.	7 July	0.78*	0.07	0.1
<i>Mertensia ciliata</i> (James) G. Don	9 July	0.43*	0.17	14.0*
<i>Linum lewisii</i> Pursh	17 July	0.64*	-0.10	-0.3
<i>Potentilla hippiana</i> Lehm. × <i>P.</i> <i>gracilis</i> Douglas	21 July	0.66*	0.12	1.1
<i>Collomia linearis</i> Nutt.	24 July	0.22	-0.27	-0.3
<i>Delphinium barbeyi</i> Huth	24 July	0.49*	0.14*	22.0*

Appendix B

Coordinates and peak flowering dates of *Mertensia fusiformis* for all study sites used for work described in Chapter 4.

Site pair	Early/late	Latitude (N)	Longitude (W)	Elevation	Peak
BC	early	38° 51' 39.0"	106° 55' 13.0"	2740	17 May
	late	38° 52' 44.5"	106° 53' 52.8"	2755	2 June
MCB	early	38° 53' 19.5"	106° 57' 43.4"	2882	20 May
	late	38° 54' 16.0"	106° 56' 35.0"	3068	7 June
401	early	38° 57' 41.3"	106° 59' 4.5"	2981	26 May
	late	38° 58' 2.5"	106° 59' 25.0"	3009	7 June
SG	early	38° 57' 15.2"	106° 59' 2.9"	2935	28 May
	late	38° 57' 18.4"	106° 59' 4.4"	2923	24 June
AV	early	38° 58' 16.4"	106° 59' 43.9"	2957	1 June
	late	38° 58' 29.0"	106° 59' 45.8"	2933	16 June
IT	early	38° 51' 19.9"	107° 5' 22.3"	3005	6 June
	late	38° 51' 16.6"	107° 5' 32.4"	2994	20 June
SP	early	38° 51' 23.1"	107° 4' 21.2"	2963	7 June
	late	38° 51' 19.4"	107° 4' 18.0"	2958	18 June

Appendix C

Taxa recorded visiting flowers of *Mertensia fusiformis* in Gunnison County, Colorado, USA (2006–2010).

Order	Family	Species
Diptera		
	Anthomyiidae	
		<i>Pegohylemyia</i> sp.
	Bombyliidae	
		Bombyliinae sp.
	Syrphidae	
		<i>Eupeodes (Eupeodes)</i> sp.
		<i>Platycheirus</i> sp.
		<i>Sphaerophoria</i> sp.
Hymenoptera		
	Apidae	
		<i>Bombus appositus</i> Cresson
		<i>Bombus bifarius</i> Cresson
		<i>Bombus californicus</i> Smith
		<i>Bombus centralis</i> Cresson
		<i>Bombus flavifrons</i> Cresson
		<i>Bombus frigidus</i> Smith
		<i>Bombus melanopygus</i> Nylander
		<i>Bombus mixtus</i> Cresson
		<i>Bombus nevadensis</i> Cresson
		<i>Bombus sylvicola</i> Kirby
	Colletidae	
		<i>Colletes</i> sp.
	Halictidae	
		<i>Halictus virgatellus</i> Cockerell
		<i>Lasioglossum (Dialictus)</i> sp.
		<i>Lasioglossum (Evylaeus)</i> sp.

Order	Family	Species
Hymenoptera (<i>continued</i>)		
	Megachilidae	
		<i>Osmia albolateralis</i> Cockerell
		<i>Osmia bucephala</i> Cresson
		<i>Osmia ednae</i> Cockerell
		<i>Osmia iridis</i> Cockerell & Titus
		<i>Osmia paradisica</i> Sandhouse
		<i>Osmia pentstemonis</i> Cockerell
		<i>Osmia proxima</i> Cresson
		<i>Osmia sculleni</i> Sandhouse
		<i>Osmia simillima</i> Smith
		<i>Osmia tersula</i> Cockerell
		<i>Osmia tristella</i> Cockerell
Lepidoptera		
	Hesperiidae	
		Hesperiidae sp.
	Pieridae	
		Pieridae sp.

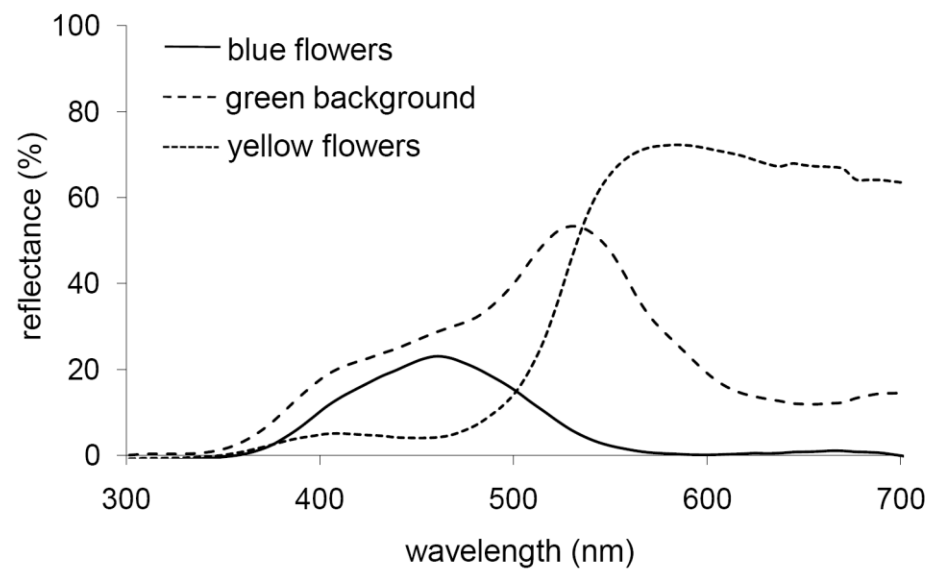
Appendix D

Numbers of all species of Hymenoptera (excluding Ichneumonoidea) emerging from trap-nests in 2008–2010. Insects emerging in 2008 were from nests constructed in 2007; insects emerging in 2009 and 2010 were from nests constructed in 2008. Species documented only in 2010 likely have an obligate 2-year life cycle, whereas those that emerged in both 2010 and one of the previous years are parsvoltine and have both 1- and 2-year forms.

Family	Species	Emerged in:		
		2008	2009	2010
Chrysididae				
	<i>Chrysis coerulans</i> Fabricius	14	66	
	<i>Omalus aeneus</i> (Fabricius)	10	7	
	<i>Trichrysis doriae</i> (Gribodo)	2		
Colletidae				
	<i>Hylaeus annulatus</i> (Linnaeus)	68	3	
	<i>Hylaeus verticalis</i> (Cresson)	1		
Gasteruptiidae				
	<i>Gasteruption kirbii</i> (Westwood)	47	8	
Megachilidae				
	<i>Coelioxys funeraria</i> Smith		7	
	<i>Coelioxys moesta</i> Cresson		48	
	<i>Hoplitis albifrons</i> (Kirby)	6	22	
	<i>Hoplitis fulgida</i> (Cresson)	88	54	
	<i>Hoplitis robusta</i> (Nylander)	36		
	<i>Megachile relativa</i> Cresson	2	205	
	<i>Osmia bucephala</i> Cresson		1	1
	<i>Osmia coloradensis</i> Cresson			49
	<i>Osmia iridis</i> Cockerell & Titus	5	37	518
	<i>Osmia lignaria</i> Say	19		
	<i>Osmia montana</i> Cresson			2

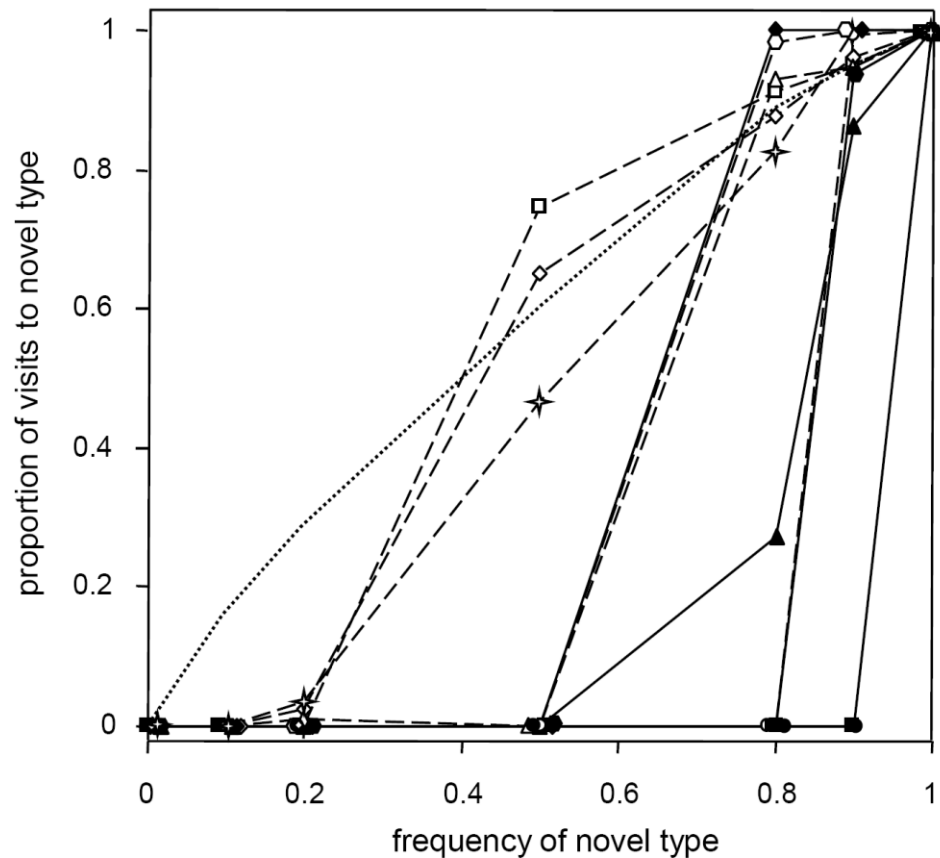
Family	Species	Emerged in:		
		2008	2009	2010
Megachilidae, <i>continued</i>				
	<i>Osmia pikei</i> Cockerell	13	33	
	<i>Osmia subaustralis</i> Cockerell			26
	<i>Osmia tersula</i> Cockerell	4	1	154
	<i>Osmia tristella</i> Cockerell	6		110
	<i>Stelis montana</i> Cresson	9	29	
	<i>Stelis submarginata</i> Cresson		3	
Sapygidae				
	<i>Sapyga pumila</i> Cresson		2	102
	<i>Sapyga</i> sp.	1		
Sphecidae				
	<i>Passaloecus borealis</i> Dahlbom		1	
	<i>Passaloecus cuspidatus</i> Smith	8	28	
	<i>Passaloecus monilicornis</i> Dahlbom	2		
	<i>Trypoxylon frigidum</i> (Smith)	14	6	
Vespidae				
	<i>Ancistrocerus albolacteus</i> Bequaert	6	13	
	<i>Ancistrocerus albophaleratus</i> (de Saussure)	12	59	
	<i>Ancistrocerus antilope</i> (Panzer)	6	39	
	<i>Ancistrocerus</i> sp. "B"	1	1	
	<i>Ancistrocerus</i> sp. nr. <i>albophaleratus</i>		6	
	<i>Euodynerus leucomelas</i>		1	
	<i>Symmorphus albomarginatus</i> (de Saussure)		6	
	<i>Symmorphus cristatus</i> (de Saussure)	32	45	

Appendix E



Reflectance spectra for artificial flowers and background colours used in the bumble bee foraging experiments, obtained with an Ocean Optics USB2000 spectrometer.

Appendix F



Proportion of bumble bee visits received by artificial flowers of the novel colour, according to their frequency in the array, with values for each individual bee shown by a separate line. Open symbols and dashed lines represent bees for which blue was the novel colour ($N = 6$); shaded symbols and solid lines represent bees for which yellow was novel ($N = 6$). Points lying above the dotted line suggest a preference for the novel colour (*i.e.*, a significant departure from a 50:50 expectation, based on a χ^2 test with a total of 100 visits). Some points are offset for clarity.

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